Charles University, Faculty of Science
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Implication of snow leopard distribution, population dynamics and landscape genetics, and prey preference for its conservation in Nepal

Vliv rozšíření, populační dynamiky a krajinné genetiky, a preference druhů kořisti na ochranu levharta sněžného v Nepálu

Ph.D Thesis

Supervisor: Prof. RNDr. Pavel Kindlmann, DrSc.

Prague, 2021

DECLARATION:

I hereby declare that this Ph.D. thesis is exclusively my own work, and that it has not been submitted (or any of its part) in order to obtain any academic degree earlier or at another institution. All publications and other sources used in the thesis have been properly quoted.

PROHLÁŠENÍ:

Prohlašuji že jsem závěrečnou práci zpracoval samostatně a že jsem uvedl všechny použité informační zdroje a literaturu. Tato práce ani její podstatná část nebyla předložena k získání jiného nebo stejného akademického titulu.

V Praze 25.01.2021 Bikram Shrestha

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Paper II **Shrestha B,** Sherchan AM, Joshi J, Karmacharya D, Kindlmann P (accepted) Non-Invasive genetic population survey of snow leopard and its co-predators in Annapurna and Sagarmatha region of Nepal. In: Kindlmann P. (ed.) Population dynamics of snow leopard, Springer, Dordrecht, in press.

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Paper VI **Shrestha B**, Aihartza J, Kindlmann P (2018) Diet and prey selection by snow leopards in the Nepalese Himalayas. PLoS ONE 13(12):e0206310. https://doi.org/10.1371/journal.pone.0206310. (IF= 2.776) (2018).

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AUTHOR'S CONTRIBUTIONS

Paper I: BS collected field data and samples, performed MaxEnt and analysis, prepared habitat suitability map and led writing of the paper.

Paper II: BS collected field data and samples, analysed the data and led writing of the paper.

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Paper IV: B.S. collected field data and samples, involved in data analysis with genetic and GIS technical assistance team and led writing of the paper.

Paper V: BS collected field data and samples, analysed the data and led writing of the paper.

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Prof. RNDr. Pavel Kindlmann, DrSc.

ABSTRACT

This thesis deals with an endangered large mammal species - snow leopard, its distribution, population dynamics, landscape genetics and connectivity, trophic ecology and human-snow leopard conflicts in the Nepalese Himalaya (Sagarmatha National Park (SNP), Lower Mustang (LM) and Upper Manang (UM) in the Annapurna Conservation Area, during Wet and Dry seasons in 2014–2016. In the case of snow leopard study, we used data obtained from camera traps, scat's genetic analysis and monitoring of fresh pugmarks and scrapes while direct count method was used to study for its main prey, blue sheep and Himalayan tahr.

In **study 1**, we assessed the determinants of habitat suitability of snow leopards using MaxEnt model and mapped the distribution of suitable habitat for snow leopards in Nepal. Altitude and Annual mean temperature are important common factors contributing to snow leopard habitat suitability within the area studied, which is indicated by both the percentage contribution of environmental variables and Jackknife test from MaxEnt model. Some other uncommon factors also seem to play a role, as they were important in at least one of the analyses. These were: distance from road, and precipitation of driest month but their importance has to be considered with caution.

In **study 2,** we present our observations along with other published data on population abundance and trend in changes of population sizes of snow leopard and its main prey, Himalayan tahr and blue sheep in the three study areas. Additionally, population data of the main leopard prey (sex ratio, female to cub ratio), wherever these values were available, are also presented. The basic analyses of these data performed yield predictions useful for developing of effective snow leopard management strategies.

In **study 3**, we use our data collected in Nepal to determine the areas suitable for snow leopards, by using habitat suitability maps, and describe the genetic structure of the snow leopard within and between these areas. We also determine the influence of landscape features on the genetic structure of its populations and reveal corridors connecting suitable areas. We conclude that it is necessary to protect these natural corridors to maintain the possibility of snow leopards' migration between suitable areas, which will enable gene flow between the diminishing populations and thus maintain a viable metapopulation of snow leopards.

In **study 4,** we studied diet and prey preference of snow leopards in the three studied areas. We collected 268 scats along 139.3 km linear transects, of which 122 were genetically confirmed to belong to snow leopards. Their diet was identified by comparing hairs in scats with our reference collection of the hairs of potential prey. In the SNP, the most frequent prey in snow leopard faeces was the Himalayan tahr in both winter and summer. In LM and UM, its main prey was blue sheep in winter, but yak and goat in summer. In terms of relative biomass consumed, yak was the main prey everywhere in both seasons. Snow leopards preferred large prey and avoided small prey in summer but not in winter, with regional differences. It preferred domestic to wild prey only in winter, and in SNP. We show that snow leopards consume a diverse range of prey, which varies both regionally and seasonally. We conclude that in order to conserve snow leopards it is also necessary to conserve its main wild species of prey, which will reduce the incidence of losses of livestock.

In **study 5,** we assess how the knowledge and perception of local people of snow leopard depredation has changed over time and its correlation with livestock losses in the central and north-eastern Himalayas in Nepal. We conclude that there is still a major threat to the long-term survival of snow leopards and its natural prey in the areas studied. Mitigation measures identified during discussions with local people should be applied to create a win-win situation for both local people and the long-term survival of snow leopards.

ABSTRAKT

Tato práce se zabývá ohroženým velkým druhem savců - sněžným levhartem, jeho rozšířením, populační dynamikou, genetikou a konektivitou krajiny, trofickou ekologií a konflikty sněžným levhartem a člověkem v nepálském Himálaji: Národní park Sagarmatha (SNP), Dolní Mustang (LM) a Upper Manang (UM) v Annapurna Conservation Area, během let 2014–2016. V případě studie levhartů sněžných jsme použili údaje získané z fotopastí, genetické analýzy trusu a sledování čerstvých pobytových známek a škrábanců, zatímco přímé odpočty byly použity ke studiu hlavní kořisti, nahura modrého a tahra himálajského.

Ve **studii 1** jsme pomocí modelu MaxEnt hodnotili determinanty vhodnosti stanovišť levhartů sněžných a mapovali rozšíření vhodného prostředí pro levharty sněžné v Nepálu. Nadmořská výška a roční průměrná teplota jsou důležitými společnými faktory, které přispívají k vhodnosti stanoviště levharta sněžného ve studované oblasti, což je indikováno jak procentním příspěvkem proměnných prostředí, tak testem Jackknife z modelu MaxEnt. Zdá se, že tu hrají roli i některé další neobvyklé faktory, které byly důležité alespoň v jedné z analýz. Byly to: vzdálenost od silnice a srážky v nejsušším měsíci, ale jejich důležitost je třeba zvažovat opatrně.

Ve **studii 2** prezentujeme naše pozorování spolu s dalšími publikovanými údaji o početnosti populace a trendu ve změnách populačních velikostí sněžného levharta a jeho hlavní kořisti, tahra himálajského a nahura modrého ve třech studovaných oblastech. Kromě toho jsou také prezentovány údaje o populaci hlavní kořisti levharta (poměr pohlaví, poměr počtů samic k počtu mláďat), pokud byly tyto hodnoty k dispozici. Základní analýzy těchto dat nám poskytly predikce užitečné pro vývoj efektivních strategií řízení levhartů sněžných.

Ve **studii 3** používáme naše údaje shromážděné v Nepálu k určení oblastí vhodných pro levharty sněžné pomocí map vhodnosti stanovišť a k popisu genetické struktury sněžného levharta v těchto oblastech a mezi nimi. Rovněž určujeme vliv krajinných prvků na genetickou strukturu jeho populací a odhalujeme koridory spojující vhodné oblasti. Dospěli jsme k závěru, že je nutné chránit tyto přirozené koridory, aby byla zachována možnost migrace levhartů sněžných mezi vhodnými oblastmi, což umožní tok genů mezi ubývajícími populacemi a tím bude udržována životaschopná metapopulace levhartů sněžných.

Ve **studii 4** jsme studovali potravní a kořistní preference levhartů sněžných ve třech studovaných oblastech. Shromáždili jsme 268 vzorků trusu podél 139,3 km lineárních transektů, z nichž 122 bylo geneticky potvrzeno, že patří k levhartům sněžným. Potrava levharta byla identifikována porovnáním chlupů v trusu s naší referenční sbírkou chlupů potenciální kořisti. V SNP byl nejčastější kořistí ve výkalech levharta sněžného himálajský tahr v zimě i v létě. V LM a UM byl jeho hlavní kořistí v zimě nahur modrý, v létě však jak a koza. Pokud jde o relativní spotřebovanou biomasu, jak byl hlavní kořistí všude v obou ročních obdobích. Sněžní levharti upřednostňovali velkou kořist a malé kořisti se vyhýbali v létě, ale ne v zimě, s regionálními rozdíly. Levhart dával přednost domácí divoké kořisti pouze v zimě a na SNP. Ukazujeme, že sněžní levharti konzumují rozmanitou škálu kořisti, která se liší regionálně i sezónně. Dospěli jsme k závěru, že za účelem ochrany levhartů sněžných je také nutné chránit jejich hlavní divoký druh kořisti, což sníží výskyt ztrát hospodářských zvířat.

Ve **studii 5** hodnotíme, jak se v průběhu času změnily znalosti a vnímání místních lidí o plenění levhartů sněžných a jejich korelace se ztrátami hospodářských zvířat ve středním a severovýchodním Himálaji v Nepálu. Dospěli jsme k závěru, že ve studovaných oblastech stále existuje velká hrozba pro dlouhodobé přežití levhartů sněžných a jejich přirozené kořisti. Měla by být použita zmírňující opatření zjištěná během diskusí s místními lidmi, aby se vytvořila situace prospěšná pro místní obyvatele i pro dlouhodobé přežití levhartů sněžných.

1. INTRODUCTION

Snow leopard, *Panthera uncia* (Schreber 1775), *syn. Uncia uncia* is a member of the genus *Panthera* in the family *Felidae*. Snow leopards are restricted to subalpine and alpine regions in South and Central Asia in 12 countries: China, Bhutan, Nepal, India, Pakistan, Afghanistan, Tajikistan, Uzbekistan, Kyrgyzstan, Kazakhstan, Russia and Mongolia (McCarthy et al. 2017). It is whitish-grey (tinged with yellow), and patterned with dark grey rosettes and spots (McCarthy and Chapron 2003). In general, snow leopards live solitarily, though small groups of up to six snow leopards are reported (McCarthy and Chapron 2003; Valentová et al. in press).

In the 1972 IUCN's Red List of Threatened Animals, it is listed as an "endangered" species (EN) due to its small population worldwide. In 2017, its categorization was changed to "vulnerable". This down-listing in the IUCN Red List is not a demotion, but it tells us that it is still endangered and in need of wide-range population monitoring and conservation (Mallon and Jackson 2017). Snow leopard is also included in Appendix I of CITES, with a minimum global population of 4,000, with 2,710–3,386 mature individuals (McCarthy et al. 2017; Valentová et al. in press). Based on the recent review of metadata (Valentová et al. in press), the total size of its current potential range is approximately 1,738,700 km^2 and the total population is between 4,501 to 6,430.

Distribution and habitat suitability

Habitat suitability is defined in terms of a habitat's potential to support a particular species and habitat suitability index is a numerical index, which ranges from a completely unsuitable habitat to an optimal habitat (Kellener 1992). This reflects spatial variability in the probability of species occurrence (Bacon et al. 2017). Habitat suitability models are used to predict species occurrence through modelling of proper environmental variables and are increasingly applied to wildlife management issues (Ottaviani et al 2004; Elith et al. 2006; Hirzelet al. 2006). Spatial structure and configuration of landscapes may have profound impacts on population distribution of a species (Collinge 2010) and habitat-based conservation measures help to keep populations at viable sizes for a longer period (Paper – I).

So far, variety of techniques and statistical methods have been used for the species distribution modelling (Corsi et al. 2000; Elith et al. 2006; Guisan and Zimmerman 2000; Scott et al. 2002; Franklin 2009). They are based on resource selection function (Boyce and McDonald 1999; Manly et al. 2002), generalized linear models (McCullagh and Nelder 1989), algorithmic modelling based on machine learning (Ripley 1996) and maximum entropy model called MaxEnt (Phillips et al. 2006). Among them, currently the most often used method is MaxEnt, because of its efficiency to handle complex interactions between response and predictor variables, and possibility to be integrated with GIS techniques (Elith et al. 2006; Yi et al. 2016) ((Paper – I).

The first preliminary habitat suitability study of snow leopard in Nepal was done by Hunter and Jackson (1997). It was based on polygon digitized expert-based model. Another suitability map was prepared using occurrence points of scats, pugmarks and scrapes, and the model was based on resource selection factor available by GIS techniques (WWF-Nepal 2009). However, molecular analyses revealed that as much as 50% of the scats collected, which look like snow leopard scats, in fact belong to other carnivores like wolf, common leopard, red fox and golden jackal (Shrestha et al. 2018). The third study was done by Aryal et al. (2016), who

estimated habitat suitability using MaxEnt model with 364 occurrences obtained mainly from few areas (Paper – I).

Habitat suitability area and spatial pattern proposed by these studies are strongly different. For example, in the Sagarmatha National Park, neither its northern part, nor its high mountains, nor its deep river gorges were reported as suitable habitats by WWF-Nepal (2009), but the whole Sagarmatha National Park, including large areas outside the park, were reported as suitable habitats by Aryal et al. (2016). Generally, one has to be very cautious in determining the suitable habitat, as even tiny parts of unsuitable habitat (high mountain peaks, deep gorges etc.) within a large area of suitable habitat can negatively affect the movement of snow leopard between patches of suitable habitat (Paper – I). Bearing in mind all these discrepancies, one has to conclude that the area of the suitability habitat of snow leopard in Nepal has still to be determined and refined (Paper – I).

Abundance, population size and population dynamics of snow leopard and its prey species

Because of the rugged and practically inaccessible terrain inhabited by snow leopards, its elusive nature and low population density, very little information is available on its distribution and population status (Jackson et al. 2006). Thus, an effective, scientific and standardized method is needed to supplement conventional techniques like surveys of signs of their presence, and so ensure the long-term survival of this endangered felid. Promising scientific techniques such as non-invasive genetic sampling and molecular scatology may yield realistic population estimates of snow leopards when complemented with information obtained using conventional techniques (Waits and Paetkau 2005; Waits et al. 2007; Broquet et al. 2007; Janecka et al. 2008). The benefit of non-invasive genetic sampling is that the target species never has to be directly observed or handled, as the most common samples used in this case are hairs and scats (Caragiulo et al. 2016). However, when collecting scat of a focal species like snow leopard in the field it is likely it will be misidentified as being scat of another species. Therefore, genetic analysis of DNA, extracted from scat, is needed to accurately identify whether it is scat of snow leopard or its co-predators (Paper – II).

The Sagarmatha National Park (SNP) is one of the most pressing regions regarding snow leopard survival. Because of adequate protection measures, snow leopards have returned to the SNP after forty years gap of its occurrence (Shrestha 2004 and 2006; Ale and Boesi 2005). For assuring its survival there, a number of questions raises. In this thesis, we will address the following ones: (i) What are the expected trends in population size and demographic structure of both snow leopard and its prey – Himalayan tahr – in the Sagarmatha region? (ii) Is snow leopard population in the SNP viable over a long run? (iii) Will the existing Himalayan tahr population be able to provide enough food for the snow leopard population of sufficient size to survive? (iv) What are the answers to these questions, when other areas of Nepal are considered? (Paper – III).

Data on population size of Himalayan tahr from 1989 and 2010 in SNP have already been presented (Shrestha 2004 and 2006; Ale 2007; Lovari et al. 2009; Ferretti et al. 2014). However, the variability in mortality rate of different sex age classes of Himalayan tahr, and relation between snow leopard density and reproductive rate of Himalayan tahr are still

missing. Better understanding of seasonal population dynamics is vital for predicting the impact of snow leopard density on Himalayan tahr or relationship between predator and prey (Paper – III).

Similarly, snow leopards have been reported in Lower Mustang and Upper Manang of ACA in several studies (Oli 1994; Ale et al. 2014) and in other areas of ACA (Aryal et al. 2014; Wegge et al. 2012), but a detailed knowledge of population trends in successive years is still lacking. We know virtually nothing about the perspectives of this species long-term survival. Long-term monitoring of snow leopard distribution patterns and demographic parameters, such as abundance, survival and recruitment, is fundamental for answering these questions. However, until now, such estimates have been based on indirect index methods, such as sign encounter rates, because of snow leopard's highly elusive behaviour, rugged landscapes forming its territory, and because of limited resources and lack of manpower necessary for collection of such data. Therefore, data available on snow leopard biology are scant, biased, and outdated and generally lack the scientific rigor (Fox et al. 1991; Jackson and Hunter 1996; Jackson et al. 2006) ((Paper – III).

Landscape genetics and connectivity

Snow leopard tends to inhabit areas with a particular type of habitat. Within these areas, adult snow leopards generally occur solitarily (Jackson and Ahlborn 1989; McCarthy et al. 2005). Between such areas, snow leopards are recorded only occasionally and are then moving between suitable areas. Based on data on its occurrence, a good and reliable map of the snow leopard distribution and migration can be produced by using habitat suitability models. Such maps act as the main components for good management plans. Migration between suitable areas may result in inbreeding (Laikre 1999), fragmentation of populations (Randi 2003), reduction in genetic variation due to habitat fragmentation, loss of connectivity (Dixon et al 2007), bottlenecks or genetic drift (Fauvergue et al. 2012). Conservation genetics then reveals the key factors, which may cause snow leopard extinction in such situations (Caragiulo et al. 2016). Thus both population genetics and habitat suitability models are crucial for designing proper management plans for its conservation (Li et al. 2016; Riordan et al. 2015; Li et al. 2020) (Paper – IV).

After the snow leopard-specific primers have been developed that enable to amplify mitochondrial DNA and polymorphic microsatellite loci (Zhang et al. 2007; Janecka et al. 2008), it became popular to identify many characteristics based on genetic studies. This included determination of species, sex, and individuals, and estimation of population size and density (McCarthy et al. 2008; Lovari et al. 2009; Janecka et al. 2011; Karmacharya et al. 2011; Wegge et al. 2012; Chetri et al. 2019), determination of genetic diversity at microsatellite loci (Janecka et al. 2008; Karmacharya et al. 2011), identification of phylogeography and genetic structure in the global range (Janecka et al. 2017) etc. However, studying only population genetic structure by itself is not sufficient. In Nepal, only one study (Karmacharya et al. 2011) analysed descriptive genetic diversity, and none exists on spatial population genetic structure based on Bayesian clustering, which is crucial for management plans aiming at snow leopard long-term persistence (Caragiulo et al. 2016) (Paper – IV).

The decline in the area of suitable habitat due to human activities and global change will make migration between such areas increasingly difficult (McCarthy and Mallon 2016; McCarthy et al. 2003; McCarthy et al. 2017). To assure the future of snow leopards, it is necessary to safeguard the survival of this species as a metapopulation, which includes maintaining the connectivity between such areas (Li et al. 2016; Riordan et al. 2015; Li et al. 2020), as only this will preserve its genetic variability (Janecka et al. 2017). Although some studies on habitat suitability were performed in Nepal (Hunter and Jackson 1997; WWF-Nepal 2009; Aryal et al. 2016) and in Tibet (Li 2013; Bai et al. 2018), the connectivity between them has never been analysed (Paper – IV).

We also need to know, how the populations differ genetically within and between such areas, in order to determine, which areas host rare genotypes and are therefore especially worthy of preservation (Randi 2003; Dixon et al. 2007; Fauvergue et al. 2012) (Paper – IV).

Diet and prey preference

Large carnivores (lion, tiger, common and snow leopard etc.) are often at risk of extinction. This, together with their visual attractiveness for people, makes them flagship species in conservation biology. Their hunting behaviour and patterns in their selection of prey may profoundly affect the population dynamics of their prey (Sih et al 1998; Miller et al. 2001; Ripple et al. 2014), which in turn affects the population dynamics of these large carnivores. In addition, if large carnivores attack domestic animals, the local people turn against them and retaliate, which causes a mixture of both positive and negative indirect interactions between wild prey on one side and cattle and other domestic animals on the other side, all sharing a common predator (Sundararaj et al. 2012; Dorresteijn et al. 2015; Ogutu et al. 2017). Therefore, an understanding of the trophic ecology and foraging strategies of large carnivores is important for predicting their population dynamics and developing effective conservation programmes (Paper – V).

A single snow leopard requires about 1.5 kg of meat per day, which is equivalent to 20–30 adult blue sheep per year (Schaller et al. 1988; Fox et al. 1989; Jackson et al. 1998). Snow leopard diet is determined by analysing the remnants of prey in their scats (Chundawat et al. 1992; Oli et al. 1993; Bagchi et al. 2006; Shrestha 2008; Lovari et al. 2009; Shehzad et al. 2012; Wegge et al. 2012; Prasad et al. 2013; Jumabay-Uulu et al. 2013; Aryal et al. 2014; Lyngdoh et al. 2014). These analyses indicate that snow leopards mainly eat ungulates (*Ovis* spp. and *Capra* spp.), but will also eat smaller prey such as marmot (*Marmota* spp.), hare (*Lepus* spp.) and/or pika (*Ochotona* spp.). According to these analyses, wild ungulates constitute the main part of the diet of snow leopard (25–90%), followed by livestock (0–67%) and smaller prey such as rodents and birds (1–40%) ((Paper – V).

However, most of the data used in these analyses are not reliable for two reasons, as is explained below. The first reason is that scats of sympatric carnivores, like wolf, common leopard, red fox, golden jackal etc., may be very similar and therefore prone to misidentification. Actually, DNA analyses of snow leopard scats confirms the visual identification in only 40–60% of the cases (Anwar et al. 2011; Janecka et al. 2011; Shehzad et al. 2012; Jumabay-Uulu et al. 2013), which suggests that the results of diet analyses obtained using conventional methods may be strongly biased. When the scats are not genetically

identified, the results are biased towards other species such as marmots (*Marmota* spp.) or bharal (*Pseudois nayaur*) (Lyngdoh et al. 2014; Lovari et al. 2013), but when identified using genetic markers, a much larger percentage of large–bodied ungulate prey is recorded (Anwar et al. 2011; Shehzad et al. 2012; Jumabay-Uulu et al. 2013) (Paper – V).

The second reason is that of the studies on snow leopard foraging behaviour, only those of Lyngdoh et al. (2014) and Chetri et al. (2017) compare snow leopard diet with prey availability, and many other studies either lack information on prey abundance (Oli et al. 1993; Chundawat et al. 1994; Prasad et al. 2013; Aryal et al. 2014) or provide data for only the largest prey available or data from a single season (Bagchi et al. 2006; Shrestha 2008; Wegge et al. 2012; Prasad et al. 2013; Jumabay-Uulu et al. 2013). However, as the gut contents reflects both availability and the predator's preference for different species of prey, these two factors must be strictly distinguished (Hayward et al. 2006). Here we take into account both of the above-mentioned reasons for possible bias (Paper – V).

Human-snow leopard conflict

In the Himalayas above 3,000 m, where the dominant vegetation is grass and alpine shrubs the majority of the people there are still dependent on animal husbandry for their livelihood (Miller 1995; Richard et al. 2000; Gurung and McVeigh 2002; McVeigh 2004), especially in areas away from trekking routes. Livestock is important in their day-to-day life and culture and livestock herding is their main economic activity, as they have limited livelihood opportunities due to shortness of the summer season, which limits them to one crop per year (MOAC 2011). However, livestock and snow leopard share the same habitat. Because of this, snow leopards frequently encounter and kill livestock and this can have a substantial effect on the local economy (Oli et al. 1994; Jackson et al. 1996; Bagchi and Mishra 2006; Shrestha 2006; Shrestha et al. 2012; Li et al. 2013; Suryawanshi et al. 2013; Ale et al. 2014; Alexander et al. 2015). Depredation of livestock is a major problem in the Himalayan region and is often reported in the local press (Fig. 8.1). The loss of livestock due to attacks by snow leopards has resulted in them being viewed as vermin that need to be eradicated (Din et al. 2017). Understanding the predation pressure on livestock and the existing ecological and social issues associated with human-snow leopard conflicts is important for developing effective means of managing and conserving large carnivores in habitats used for livestock grazing (Bagchi and Mishra 2006) (Paper – VII).

The incidence of killing, however, is highly site dependent, differs from site to site, between years, with changes in the pattern of livestock herding, and the density and behaviour of snow leopards (Jackson 2010; Suryawanshi et al. 2013). It is therefore urgent to study, how the conflict between local people and snow leopards is changing over time, and determine the major factor responsible for heightening this problem (Paper – VII).

2. MAIN QUESTIONS AND HYPOTHESES

Following the knowledge gaps identified above, I focused my research within this PhD thesis on investigating five main topics.

First, we aim to determine determinants and distribution of habitat suitability of snow leopard in Nepal (Paper I). Second, we present our observations along with other published data on population abundance and trend of snow leopard and its main prey, Himalayan tahr and blue sheep (Papers II and III). Third, we aim to identify suitable habitats for snow leopards in Nepal, connectivity between them by finding realistic corridors connecting them, and describe the genetic structure of the snow leopard within and between the areas studied (Paper IV). Fourth, we determined the diet and prey selection by snow leopards in the Nepalese Himalayas. We ask, whether snow leopards: i) consume prey in direct proportion to its availability, ii) show any selection regarding prey size, iii) distinguish between wild and domestic prey and iv) show seasonal or regional differences in their selection of prey (Papers V and VII). Fifth, we aim to compare the current level of predation and the conflict between snow leopards and people with that recorded two decades ago in Upper Manang and Sagarmatha National Park. We also assess the pattern in the killing of livestock in other snow leopard habitats and threat reduction assessment and effectiveness of mitigation measures (Paper VII).

3. MATERIALS AND METHODS

In **paper I,** we apply MaxEnt to the distribution data on snow leopard from Nepal based on a large set of occurrence data (450 observations) collected from a much wider range of areas (9 districts) than the previous studies. We used camera traps, scat collections and monitoring of fresh pugmarks and scrapes for snow leopard presence locations. All our data based on scats were consistently genotyped, to avoid misidentification of the species that produced them. All fresh pugmarks and scrapes were verified, whether or not they originate from snow leopard by using movement pattern of snow leopard from camera trap data.

In **paper II,** we studied the distribution and population size of snow leopards based on a genetic analysis of scat. In **paper III**, it is focusing on trends in changes of population sizes of snow leopard and its prey in Nepal in the three study areas in ACA and SNP. Scrape marks and camera traps were used for monitoring snow leopard while direct count method applied for monitoring main prey species. Transects characterized by elevation and several topographic parameters were established to cover the most of the typical biotope of snow leopard in study areas. Snow leopard and its prey were repeatedly monitored in SNP during dry seasons 2006, 2007, 2009 and 2015 and in ACA during dry season 2014 and wet season 2016. Encounter rate (sings/km) of scrapes marks are considered as the most reliable determinants for abundance indices. In all study areas, we walked and searched for prey within each valley, divided into several polygons based on physical barrier such as river, deep gorge or high mountain. We perform basic analyses of the data and derive predictions useful for developing effective snow leopard management strategies.

In **paper IV**, after performing habitat suitability modelling by using MaxEnt, we used circuit-scape theory model for the connectivity analysis. We collected 268 putative snow leopard samples of faeces, hairs and urine. From the collected samples, 6 microsatellite loci from different 6 chromosomes were selected for microsatellite-DNA sequence. After filtering

positive samples, we obtained only 63 microsat data to analysis genetic structure or genetic variability.

In **paper V**, hair identification key of Himalayan mammals of Nepal as a tool to study food habits of snow leopard was developed by preparing microscopical hair slides to identify and examine cuticular scale cast or impression, medulla and cross-section. In **paper VI,** we studied the prey of snow leopard in three Himalayan regions in Nepal (Sagarmatha National Park (SNP), Lower Mustang (LM) and Upper Manang (UM) in the Annapurna Conservation Area, during winter and summer in 2014–2016. We collected 268 scats along 139.3 km linear transects, of which 122 were genetically confirmed to belong to snow leopard. Their diet was identified by comparing hairs in scats with our reference collection of the hairs of potential prey. We determined prey availability using 32–48 camera-traps and 4,567 trap nights.

In **paper VII**, we assess the knowledge and perception of local people of livestock losses due to snow leopards in the central and north-eastern Himalayas in Nepal. In nine settlements in three protected areas (Annapurna Conservation Area – ACA, Manaslu Conservation Area – MCA and Sagarmatha National Park – SNP) we studied, how the perception of local people of snow leopard depredation has changed over time and its correlation with livestock losses. We carried out questionnaire-based interviews of 1015 households from 2004 to 2016, which included 26.45% to 100% of all households in the settlements.

4. RESULTS AND DISCUSSION

In **paper I,** altitude and annual mean temperature are important common factors contributing to snow leopard habitat suitability within the area studied indicated by both the percentage contribution of environmental variables and Jackknife test from MaxEnt model. Some other uncommon factors also seem to play a role, as they were important in at least one of the analyses. These were: distance from road, distance from roads and precipitation of driest month but their importance has to be considered with caution. To conclude: the habitat suitability models indicate that the main danger for snow leopard survival may be climate warming and human expansion. Both these phenomena will push the lower limit of its distribution upwards to higher elevations, which will entail two negative effects.

In **paper II,** after data quality filtering, 63 microsatellite genotypes were obtained, corresponding to 22 individuals according to the identity analysis. In **paper III**, our study showed that number of scrapes/km was positively correlated with snow leopard density obtained by camera trapping. With data from ACA, we found that number of scrapes recorded during dry period 2014 were significantly higher than during wet period 2016. In total 19 individuals of snow leopards were identified by camera trap in three studied areas. There was no significant trend in population density of blue sheep either in LM or in UM. In SNP, there were some fluctuations and differences among several valleys but overall the tahr population has decreased during 1989–2015.

In **paper IV**, the connectivity maps reveal the Central and Eastern Nepal are interconnected by rather narrow corridors, which are very vulnerable, because they contain

bottlenecks. Because of high mountain barrier between the protected areas, the corridor goes partially through the Tibetan Qomolangma National Nature Reserve. Observed heterozygosity shows leopard populations have moderate genetic diversity. According to the genetic results, the genetic structures between Manang and Mustang are more similar and sufficient to prevent the effects of genetic drift in these two regions, as there are three possible corridors between them. In contrast, between Sagarmatha north (N-S) and Sagarmatha south (SW-S), there is a weak narrow belt of corridor, high deep gorge, two big river meeting between them. There is also one route between them with high tourist visitors. Because of this, snow leopard population fragmented into two sub-cluster., i.e., they do not share too much genetic diversity because of disturbance and barrier. The results of the landscape genetic analysis indicate only a fraction degree of gene flow between the two areas studied (LM & UM vs. N-S & SW-S) which is approximately 300 km apart, however snow leopard can cover even 27-40 km per day and that the average home range is 200-500 km^2.

In **paper V**, we provide a detailed microstructure characteristics and micrometres measurements from the hairs of Himalayan mammals sampled within the snow leopard habitats in Nepal. Moreover, digital photographs reference key of the medulla, cuticle (scales) and cross-sections of guard hairs with the description of hair characteristics are provided as a tool for hair identification.

In **paper VI,** in the SNP, the most frequent prey in snow leopard faeces was the Himalayan tahr in both winter and summer. In LM and UM, its main prey was blue sheep in winter, but yak and goat in summer. In terms of relative biomass consumed, yak was the main prey everywhere in both seasons. Snow leopard preferred large prey and avoided small prey in summer but not in winter, with regional differences. It preferred domestic to wild prey only in winter, and in SNP. Unlike most other studies carried out in the same area, our study uses genetic methods for identifying the source of the scat. Studies solely based on visual identification of samples may be strongly biased. Diet studies based on frequency of occurrence of prey tend to overestimate the importance of small prey, which may be consumed more often, but contribute less energy than large prey. However, even assessments based on prey biomass are unlikely to be accurate, as we do not know whether the actual size of the prey consumed corresponds to the average size used to calculate the biomass eaten. For example, large adults may be too difficult to catch and therefore mostly young animals are consumed, whose weight is much lower. We show that snow leopard consumes a diverse range of prey, which varies both regionally and seasonally. We conclude that in order to conserve snow leopards it is also necessary to conserve its main wild species of prey, which will reduce the incidence of losses of livestock.

In **paper VII**, herding of yak/nak (nak is female yak), sheep/goats and cattle (cows, oxen and horses) were found to be the main sources of livelihood for all households in the villages. Herders reported losses of livestock mainly due to attacks by snow leopard and two other carnivores (wolf and lynx). Most (1.5% to 14.3%) losses were attributed to snow leopard, while the other predators accounted for a meagre 0.16% to 5.3%. Predator-induced loss was substantial for the local families and reached $349 per household per year. However, livestock mortality due to other causes (disease or natural disasters) was higher than that attributed to predators. We also evaluated the effectiveness of existing mitigating programmes, described

community-based local mitigation measures and assessed the subsequent reduction in the level of conflict. This revealed that the number of conflicts was lower than in 1990 and 50% of the respondents had changed their mind about snow leopard conservation. Surprisingly many respondents (15%) were against the conservation of snow leopards and even considered retaliatory killing of this predator as the best solution. Of the five snow leopard-human conflict mitigation measures, compensation from a community-based livestock insurance scheme and the improving of animal husbandry were the most popular in all the regions studied. Altogether, 15 human-induced threats to the future survival of snow leopards and its wild prey were identified in two protected areas. We conclude that there is still a major threat to the long-term survival of snow leopards and its natural prey in the areas studied. Mitigation measures identified during discussions with local people should be applied to create a win-win situation for both local people and the long-term survival of snow leopards.

5. CONCLUSIONS

In the studies outline above, we attempt to gain insights into snow leopard distribution, population dynamics, landscape genetics and connectivity, trophic ecology and human-snow leopard conflicts in the Nepalese Himalaya.

The results presented in **paper I** revealed that annual mean temperature and altitude were the two main factors influencing snow leopard habitat suitability in Nepal using the MaxEnt model. As these two factors are strongly correlated, one can conclude that the main factor driving the presence of snow leopard is just one of them – biologically more meaningful is altitude. The response curve of different predictor variables of our study revealed that the probability of snow leopard presence in Nepal was highest in the altitude of around 4,000 m a.s.l. with a relatively cold and dry climate, shrubs, rocks and open grassland.

In **paper II,** we studied the distribution and population size of snow leopards based on a genetic analysis of scat. **Paper III** is focusing on trends in changes of population sizes of snow leopard and its prey in Nepal in the three study areas, Lower Mustang, Upper Manang and Sagarmatha National Park. Here we present our observations along with other published data on population abundance and trend of snow leopard and its main prey, Himalayan tahr and blue sheep. Additionally, population data of the main leopard prey (sex ratio, female to cub ratio) wherever these values were available are also presented. We perform basic analyses of these data and derive predictions useful for developing effective snow leopard management strategies.

The main message in **paper IV** is the delimitation of the areas with suitable habitat for snow leopards in Nepal and identification of the main corridors connecting these areas. We show that the genetic structure of snow leopard populations is mainly influenced by the proximity of people and trekking routes used by tourists, which pose a barrier to the dispersal of snow leopards. Topography of the terrain also plays a major role, as it determines the occurrence of suitable habitat for snow leopards in Nepal.

In **paper V**, we provide the hair identification key of Himalaya mammals of Nepal as a tool to study food habits of snow leopard. During the study for **paper VI,** we found snow

leopard consumes a diverse range of prey, which varies both regionally and seasonally. In the SNP, the most frequent species of prey was Himalayan tahr in both winter and summer, followed by cow and musk deer in winter, and cow and yak in summer; weasel spp. and dog were also consistently recorded in both seasons and some small prey occurred only in summer, whereas in the ACA, the main prey was blue sheep in winter and yak and goat in summer. Snow leopard prefers large prey and avoids small prey in summer but not in winter. In SNP, wild prey was eaten only in winter. A higher percentage of wild animals was consumed in winter (69%) and domestic animals in summer (54%). In overall (in term of frequency of occurrence of prey in the diet), snow leopard consumed 57% of wild prey and 43 % of domestic animals. In term of prey preference/selection, interestingly, livestock was preferred only in SNP throughout the year and in winter in the whole study area, precisely where and when wildlife is more frequently available.

In **paper VII**, we assess the knowledge and perception of local people of livestock losses due to snow leopards in the central and north-eastern Himalayas in Nepal. We conclude that there is still a major threat to the long-term survival of snow leopards and its natural prey in the areas studied. Mitigation measures identified during discussions with local people should be applied to create a win-win situation for both local people and the long-term survival of snow leopards.

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7. ATTACHED PUBLICATIONS

PAPER I TO VII

Paper I

Assessment of habitat suitability of snow leopard in Nepal using MaxEnt modelling

Bikram Shrestha and Pavel Kindlmann

Chapter 6: Shrestha B, Kindlmann P (accepted) Assessment of habitat suitability of snow leopard in Nepal using MaxEnt modelling. In: Kindlmann P. (ed.) Population dynamics of snow leopard, Springer, Dordrecht, in press

Abstract

Habitat suitability models are used to predict species occurrence through modelling of proper environmental variables and are increasingly applied to wildlife management issues. So far, variety of techniques and statistical methods have been used for the species distribution modelling. Here we apply MaxEnt to the distribution data on snow leopard from Nepal based on a large set of occurrence data collected from a much wider range of areas (9 districts) than the previous studies. We used camera traps, scat collections and monitoring of fresh pugmarks and scrapes. All our data based on scats were consistently genotyped, to avoid misdetrermination of the species that produced them. All fresh pugmarks and scrapes were verified, whether or not they originate from snow leopard by using movement pattern of snow leopard from camera trap data. Altitude and Annual mean temperature are important common factors contributing to snow leopard habitat suitability within the area studied indicated by both the percentage contribution of environmental variables and Jackknife test from MaxEnt model. Some other uncommon factors also seem to play a role, as they were important in at least one of the analyses. These were: distance from road, distance from roads and precipitation of driest month but their importance has to be considered with caution. To conclude: the habitat suitability models indicate that the main danger for snow leopard survival may be climate warming and human expansion. Both these phenomena will push the lower limit of its distribution upwards to higher elevations, which will entail two negative effects.

6.1 Introduction

Habitats can be defined as regions in environmental space (Aarts et al. 2008; Hirzel and Lay 2008) that are composed of biotic or abiotic environmental variables related directly (e.g. forage biomass and quality) or indirectly (e.g. elevation) to the use of a location by the animal (Beyer et al. 2010). Habitat suitability is defined in terms of a habitat's potential to support a particular species and habitat suitability index is a numerical index, which ranges from a

completely unsuitable habitat to an optimal habitat (Kellener 1992). This reflects spatial variability in the probability of species occurrence (Bacon et al. 2017). Habitat suitability models are used to predict species occurrence through modelling of proper environmental variables and are increasingly applied to wildlife management issues (Ottaviani et al 2004; Elith et al. 2006; Hirzelet al. 2006). Spatial structure and configuration of landscapes may have profound impacts on population distribution of a species (Collinge 2010) and habitat-based conservation measures help to keep populations at viable sizes for a longer period.

During the last few decades, species distribution models became widely used to quantify animal-habitat relationships, to describe and predict differential space use by animals, to identify habitat that is important to an animal (Shrestha 2006; Beyer et al. 2010) and for estimating the impact of climate change on species distribution (Bakkenes et al. 2006; Lavergne et al. 2010). This is essential for effective conservation and management applications (Kie et al. 2010). Species endangerment, species extinction and biodiversity loss are associated with habitat loss or small geographical range size and low population density (Purvis et al. 2000). Therefore, information on habitat particularly important to a species is crucial to ensure that it is properly managed and to determine the priorities in protection.

So far, variety of techniques and statistical methods have been used for the species distribution modelling (Corsi et al. 2000; Elith et al. 2006; Guisan and Zimmerman 2000; Scott et al. 2002; Franklin 2009). They are based on resource selection function (Boyce and McDonald 1999; Manly et al. 2002), generalized linear models (McCullagh and Nelder 1989), algorithmic modelling based on machine learning (Ripley 1996) and maximum entropy model called MaxEnt (Phillips et al. 2006). Among them, currently the most often used method is MaxEnt, because of its efficiency to handle complex interactions between response and predictor variables, and possibility to be integrated with GIS techniques (Elith et al. 2006; Yi et al. 2016).

Here we apply MaxEnt to the distribution data on snow leopard from Nepal and adjacent parts of Tibet. Although some studies have been conducted on its habitat suitability (Fox 1994; Hunter and Jackson 1997; McCarthy et al. 2016; Holt et al. 2018; Kalashnikova et al. 2019; Watts et al. 2019; Atzeni et al. 2020) and a ranged-wide distribution (Li et al. 2016), only few such studies have been performed in the fragile ecosystems of Nepal.

The first preliminary habitat suitability study of snow leopard in Nepal was done by Hunter and Jackson (1997). It was based on polygon digitized expert-based model. Another suitability map was prepared using occurrence points of scats, pugmarks and scrapes, and the model was based on resource selection factor available by GIS techniques (WWF-Nepal 2009).

However, molecular analyses revealed that as much as 50% of the scats collected, which look like snow leopard scats, in fact belong to other carnivores like wolf, common leopard, red fox and golden jackal (Shrestha et al. 2018). The third study was done by Aryal et al. (2016), who estimated habitat suitability using MaxEnt model with 364 occurrences obtained mainly from few areas.

Habitat suitability area and spatial pattern proposed by these studies are strongly different. For example, in the Sagarmatha National Park, neither its northern part, nor its high mountains, nor its deep river gorges were reported as suitable habitats by WWF-Nepal (2009), but the whole Sagarmatha National Park, including large areas outside the park, were reported as suitable habitats by Aryal et al. (2016). Generally, one has to be very cautious in determining the suitable habitat, as even tiny parts of unsuitable habitat (high mountain peaks, deep gorges etc.) within a large area of suitable habitat can negatively affect the movement of snow leopard between patches of suitable habitat.

Bearing in mind all these discrepancies, one has to conclude that the area of the suitability habitat of snow leopard in Nepal has still to be determined and refined. Therefore, in our study, we used the MaxEnt model based on a large set of occurrence data (450 observations), collected from a much wider range of areas (9 districts) than the previous studies. The data were sampled not only from a restricted range of sites, but from across the whole Nepal. We used camera traps, scat collections and monitoring of fresh pugmarks and scrapes. All our data based on scats were consistently genotyped, to avoid misdetrermination of the species that produced them. All fresh pugmarks and scrapes were verified, whether or not they originate from snow leopard by using movement pattern of snow leopard from camera trap data.

6.2 Study Area

Habitat suitability of snow leopard was estimated in entire snow leopard range across northern Himalayan range of Nepal. Camera trap and genetic analysis from scats were conducted in Lower Mustang and Upper Mustang of Annapurna Conservation Area and Sagarmatha National Park (see chapters 5 and 9 for details on these areas).

6.3 Methodology

6.3.1 Snow Leopard Occurrence Data and Modelling

Data on occurrences were obtained by monitoring snow leopard signs transects and camera trapping, and GPS locations of genotyped scat, the collection of which is explained in chapters 5 and 9 respectively.

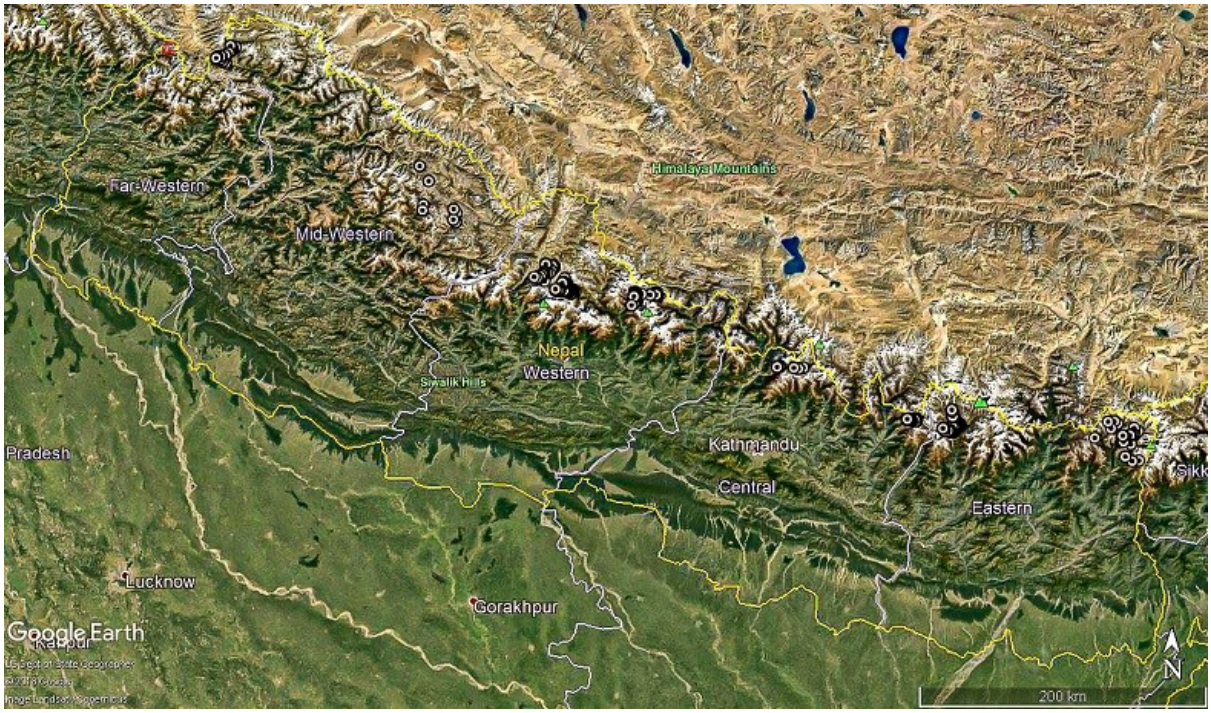

Fig. 6.1 Map of the area showing the locations where we collected samples are indicated by circles

There were 450 records of occurrence in nine different districts (Fig. 6.1), which were collected from 2004 to 2016, mostly from the Sagarmatha National Park (Fig. 6.2) and Annapurna Conservation Area (particularly Upper Manang and Lower Mustang) (Fig. 6.3 and Fig. 6.4). Some literature data were also used, like Khatiwada and Ghimirey (2008), Shrestha (2008), WWF-Nepal (2009) and CMDN (2010). For description of the environmental conditions and preparation of the habitat suitability model, habitat variables related to terrain (topography), 19 bio-climatic variables, habitat (land cover) and effect of human activity or disturbance (distance to nearest roads and buildings) were used (Table 6.1), which are likely to affect snow leopard presence/absence.

For example, the typical habitat of snow leopard is rather rough (Fig. 6.5 and Fig. 6.6). Its upper limit goes to the snow line (Fig. 6.7 and Fig. 6.8).

The total number of variables that were initially used are presented in Table 6.1.

Fig. 6.2 Snow leopard scat sample recorded by researcher Bikram Shrestha in SNP

Fig. 6.3 Snow leopard and blue sheep habitat – Upper Manang, ACA

Fig. 6.4 Snow leopard and blue sheep habitat – Lower Mustang, ACA

Fig. 6.5 Snow leopard and Himalayan tahr habitat – SNP

Fig. 6.6 Gokyo valley scanned from Renjo La pass (5,400 m a.s.l.), a typical habitat of snow leopard; Mt. Everest in the background

Fig. 6.7 After heavy snowing in 2014 during winter in Lower Mustang – ACA. Snow line goes down at ~ 4,000 m a.s.l. during winter

Fig. 6.8 Grassland in Upper Manang – ACA during summer season; snow line goes up above 5,500 m a. s. l.

Table 6.1 Environmental variables used in the study of the potential distribution of snow leopard. The codes used for variables are in parentheses

	Variable	**Source**
Topography	Altitude (Alt)	SRTM (© CGIAR-CSI, 2004)
	Ruggedness index (Ruggedness)	
	Vertical heterogeneity (Ver_het)	
	Slope	
Climate	Annual mean temperature (Bio1)	WorldClim 2.0 (Fick and Hijmans 2017)
	Mean diurnal range (Bio2)	
	Isothermality (Bio3)	
	Temperature seasonality (Bio4)	
	Temperature July (Bio5)	
	Temperature January (Bio6)	
	Temperature annual range (Bio7)	
	Mean temperature of wettest quarter (Bio8)	
	Mean temperature of driest quarter (Bio9)	
	Mean temperature of warmest quarter (Bio11)	
	Annual precipitation (Bio12)	
	Precipitation of wettest month (Bio13)	
	Precipitation of driest month (Bio14)	
	Precipitation seasonality (Bio15) (Coefficient of variation)	
	Precipitation of wettest quarter (Bio16)	
	Precipitation of driest quarter (Bio17)	

	Precipitation of warmest quarter (Bio18)	
	Precipitation of coldest quarter (Bio19)	
Habitat	Land cover main	FAO Global Land Cover Network (http://www.fao.org/geospatial-/projects/detail/en/c/1035672/)
Effect of human activity	Distance to roads (Dist_roads)	Open Street Maps (2017)
	Distance to buildings (Dist_builings)	(www.openstreetmap.org)

The raster digital elevation model (DEM) was obtained from SRTM (http://www.cgiar-csi.org/data/srtm-90m-digital-elevation-database-v4-1) in 1-sec resolution (approx. 30 m). It also generated rasters of slope and altitude. The bioclimatic variables were obtained from the WorldClim database in a 30-sec resolution (approx. 1 km^2). Land cover was obtained in vector format (polygons) from FAO Global Land Cover Network. The default 33 categories were merged into 9 main classes of land cover. Distance from roads in euclidean spatial raster in 30-m resolution generated from the line elements of the Open Street Map database. Distance from human settlements in euclidean spatial raster in 30-m resolution generated from polygonal elements of the Open Street Map database. All these layers of environmental variables were prepared in ArcMap 10.6.1. All data were converted into a uniform raster with a 100-m spatial resolution in ASCII format.

ENM Tools version 1.3 were used (Warren et al. 2010) to test for multi-collinearity between 19 bio-climatic variables. ENM Tools output matrix of Pearson Correlation Coefficients (r) (Table 6.2) was used to drop variables with $|r| \geq 0.75$. Of the correlated variables, only the variable with the higher percentage contribution and training gain was selected (Tsiftsis et al. in press). After removing 13 highly correlated variables ($|r| \geq 0.75$), the remaining six bio-climatic variables were used for producing the final habitat suitability map including other important predictable variables of altitude, ruggedness index, vertical heterogeneity, slope, land cover main, distance to roads and distance to buildings.

Models of habitat suitability indicate both the actual and potential occurrence of a focal species (Elith et al. 2006). Habitat analysis and potential distribution modelling were run using MaxEnt tool 3.4.1 (Phillips et al. 2018) and 25% of the occurrence data was used to verify the

model. Ten (10) models were run with MaxEnt using the auto-features mode and the default settings, as suggested by Phillips and Dudík (2008), bootstrapping was used as a form of replication (test samples chosen randomly by sampling with replacement). Model performance was assessed using the Akaike information criterion (AICc) as it greatly outperforms BIC and AUC based methods. Model selection was performed using ENMTools package (Warren et al. 2010) and selected the lowest AICc value (the model with the lowest AICc value is considered as the best compared to the others).

6.4 Results

6.4.1 MaxEnt Model Performance

The receiver operating characteristic (ROC) results (Fig. 6.9) of the best model showed the AUC value was high for the MaxEnt model for the training data (0.983), indicating that the predictions were excellent and potentially useful. The average training AUC for the 10 replicate runs is 0.983, and the standard deviation is 0.001. The low AUC standard deviation (0.001) suggests there was no overfitting around the presence data.

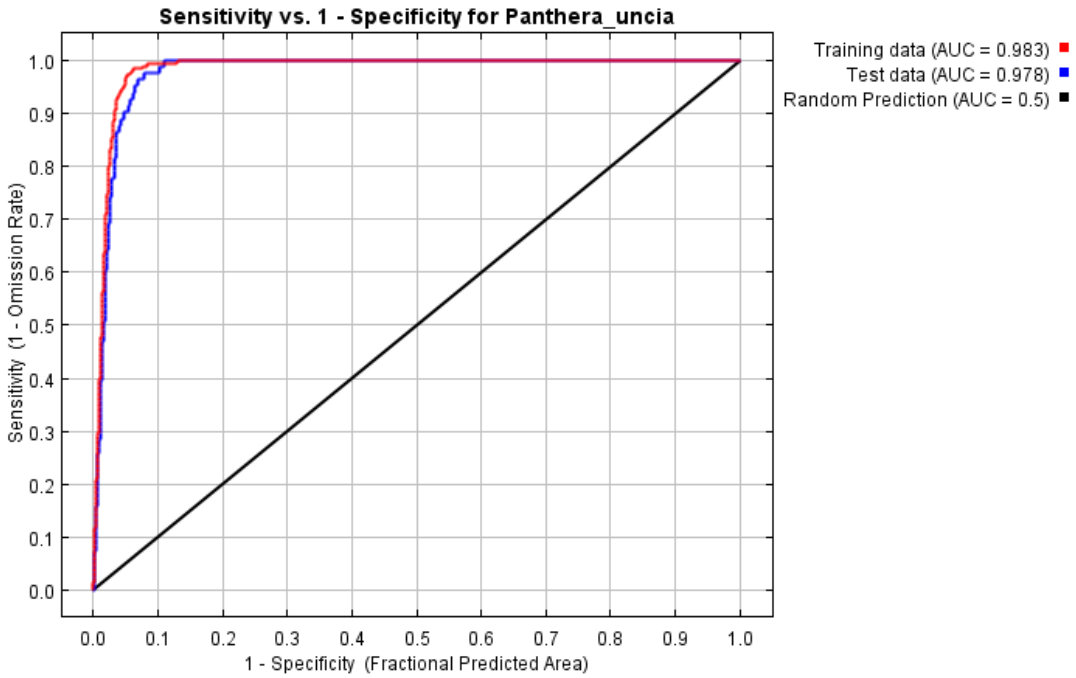

Fig. 6.9 ROC verification of distribution of suitable snow leopard habitat

Table 6.2 Pearson correlation coefficients of 19 bio-climatic variables used for snow leopard habitat suitability modelling in Nepal. $r \geq |0.75|$ are highlighted with yellow colour. Codes for variables are explained in Table 6.1 and in the text.

Variable	bio1	bio2	bio3	bio4	bio5	bio6	bio7	bio8	bio9	bio10	bio11	bio12	bio13	bio14	bio15	bio16	bio17	bio18	bio19
bio1	0.00	-0.34	0.66	-0.72	0.98	0.99	-0.68	0.92	0.92	0.99	0.99	0.81	0.86	0.04	0.76	0.85	0.29	0.67	-0.23
bio2	0.00	0.00	0.01	0.56	-0.21	-0.43	0.76	-0.38	-0.28	-0.28	-0.37	-0.64	-0.57	-0.57	-0.24	-0.57	-0.57	-0.66	-0.37
bio3	0.00	0.00	0.00	-0.80	0.61	0.69	-0.63	0.52	0.52	0.61	0.70	0.47	0.47	0.01	0.32	0.48	0.19	0.39	-0.36
bio4	0.00	0.00	0.00	0.00	-0.61	-0.80	0.96	-0.63	-0.56	-0.65	-0.79	-0.72	-0.69	-0.34	-0.38	-0.70	-0.47	-0.68	0.11
bio5	0.00	0.00	0.00	0.00	0.00	0.95	-0.55	0.91	0.93	1.00	0.97	0.74	0.81	-0.06	0.78	0.81	0.20	0.59	-0.28
bio6	0.00	0.00	0.00	0.00	0.00	0.00	-0.77	0.91	0.90	0.97	1.00	0.84	0.88	0.12	0.73	0.87	0.36	0.71	-0.19
bio7	0.00	0.00	0.00	0.00	0.00	0.00	0.00	-0.62	-0.54	-0.60	-0.74	-0.78	-0.72	-0.45	-0.38	-0.73	-0.56	-0.74	-0.06
bio8	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.79	0.92	0.91	0.78	0.83	0.12	0.82	0.82	0.30	0.67	-0.23
bio9	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.93	0.91	0.71	0.77	-0.04	0.64	0.76	0.24	0.54	-0.09
bio10	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.98	0.78	0.84	-0.01	0.78	0.83	0.25	0.63	-0.24
bio11	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.82	0.86	0.08	0.73	0.86	0.33	0.69	-0.22
bio12	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.98	0.32	0.65	0.99	0.45	0.95	0.00
bio13	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.21	0.75	1.00	0.38	0.92	-0.08
bio14	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	-0.14	0.21	0.85	0.33	0.64
bio15	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.73	0.01	0.57	-0.34
bio16	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.37	0.93	-0.09
bio17	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.37	0.71
bio18	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	-0.05
bio19	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

6.4.2 Snow leopard Suitable Habitat Distribution with Predictor Variables

The percentage contribution of environmental variables (Table 6.3) revealed the four main factors contributing to snow leopard habitat suitability within the area studied. They are distance from road (Dist_roads; 25.8%), annual mean temperature (Bio1; 23.2%), distance from buildings (Dist_buildings; 22.4%) and altitude (Altitude; 13.4%). The permutation importance in the MaxEnt model prediction (Table 6.3) indicated that annual mean temperature (Bio1; 66.4%), distance from road (Dist_roads; 8.4%), distance from buildings (Dist_buildings; 8.3%) and precipitation of driest month (Bio14; 7.8%) were the four main factors affecting snow leopard habitat preferences.

Table 6.3 Percentage contribution and permutation importance values of environmental variables (four most important factors in bold)

Variable	Percent contribution	Permutation importance
Bio1	**23.2**	**66.4**
Bio2	1.7	1.7
Bio3	2	0.2
Bio14	4.8	**7.8**
Bio18	2.6	4.4
Bio19	0.8	1.9
Dist_roads	**25.8**	**8.4**
Dist_buildings	**22.4**	**8.3**
Altitude	**13.4**	0.2
Land cover main	2.8	0.2
Vert_het	0.4	0.3
Ruggedness	0.1	0.1
Slope	0	0.1

The Jackknife test of environmental variables in training data produced by MaxEnt is presented in Fig. 6.10. From here it follows that the five main factors contributing to snow leopard habitat suitability within the area studied are altitude (Altitude), annual mean temperature (Bio1), distance from buildings (Dist_buildings), distance from road (Dist_roads) and precipitation of driest month (Bio14). The environmental variable with highest gain when used in isolation is altitude, which therefore appears to have the most useful information by itself. The environmental variable that decreases the gain the most, when omitted, distance from road (Dist_roads), which therefore appears to have the most information that is not present in the other variables. If MaxEnt uses only ruggedness index (rugged), it achieves almost no gain, so that variable is not (by itself) useful for estimating the distribution of snow leopard (Fig. 6.10).

To conclude, the common factors indicated by both the percentage contribution of environmental variables in Table 6.4 and Jackknife test in Fig. 6.3 are Altitude, Bio1, Dist_road, Dist_buildings and Bio14.

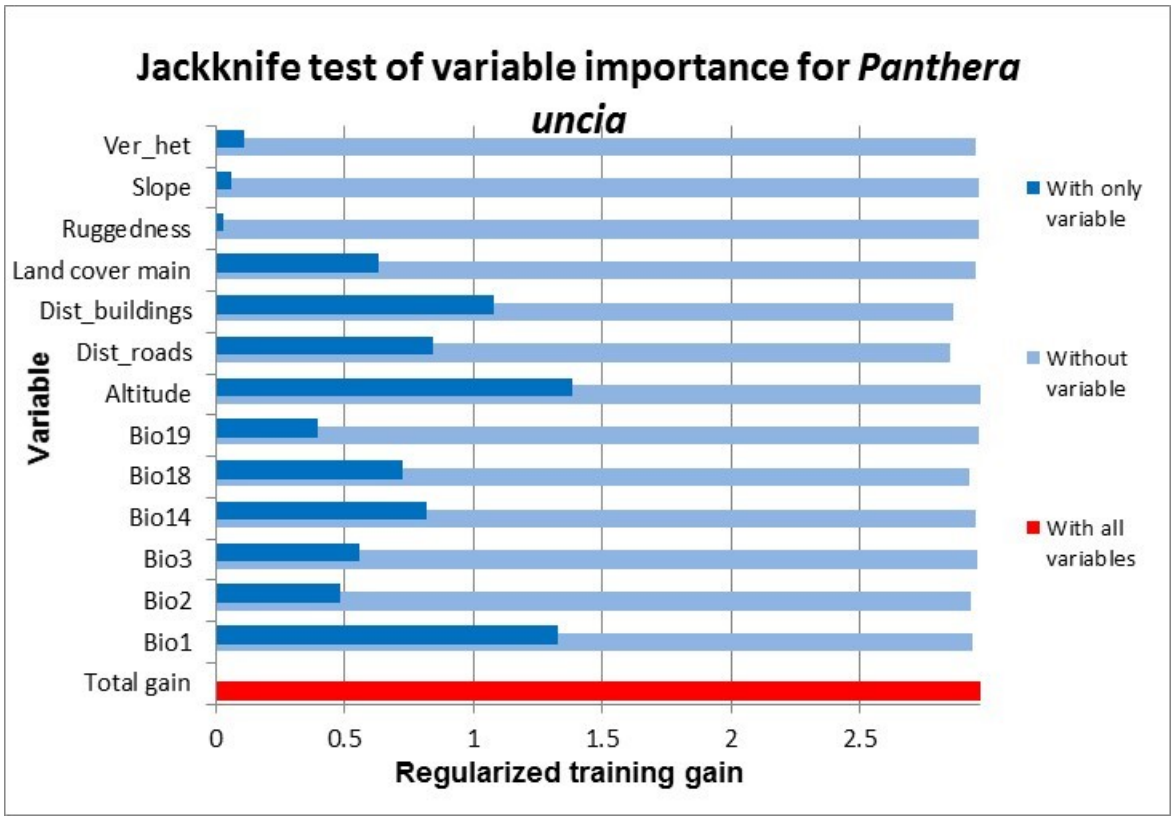

Fig. 6.10 Jackknife test of environmental variables in training data by MaxEnt

The response curves, which determine the influence of each factor on snow leopard habitat distribution are shown in Fig. 6.11. The altitude in areas suitable for snow leopard habitat was between 3,000 m and 5,000 m a.s.l, however spatial distribution of the most suitable habitats is a relatively narrow and compact belt at altitudes between ca 3,500 and 4,500 m a.s.l. as suitability habitat index was assigned more than 50% (Fig. 6.11. b). Patches of the most suitable habitat are typically southern slopes at an altitude of around 4,000 m a.s.l. with a relatively cold and dry climate, slope ranged 20^0 to 50^0, shrubs, rocks and open grassland (Fig. 6.11).

The habitat suitability map is shown in Fig. 6.12. The suitable areas are reclassified into unsuitable, low suitable and high suitable indicated by yellow, red and blue colours respectively. The belt of suitable area is widest in regions in western or central Nepal (e.g. in Annapurna and Dolpa – see Fig. 6.12). However, most of the belt in eastern Nepal is rather narrow (Fig. 6.12) and therefore vulnerable to fragmentation.

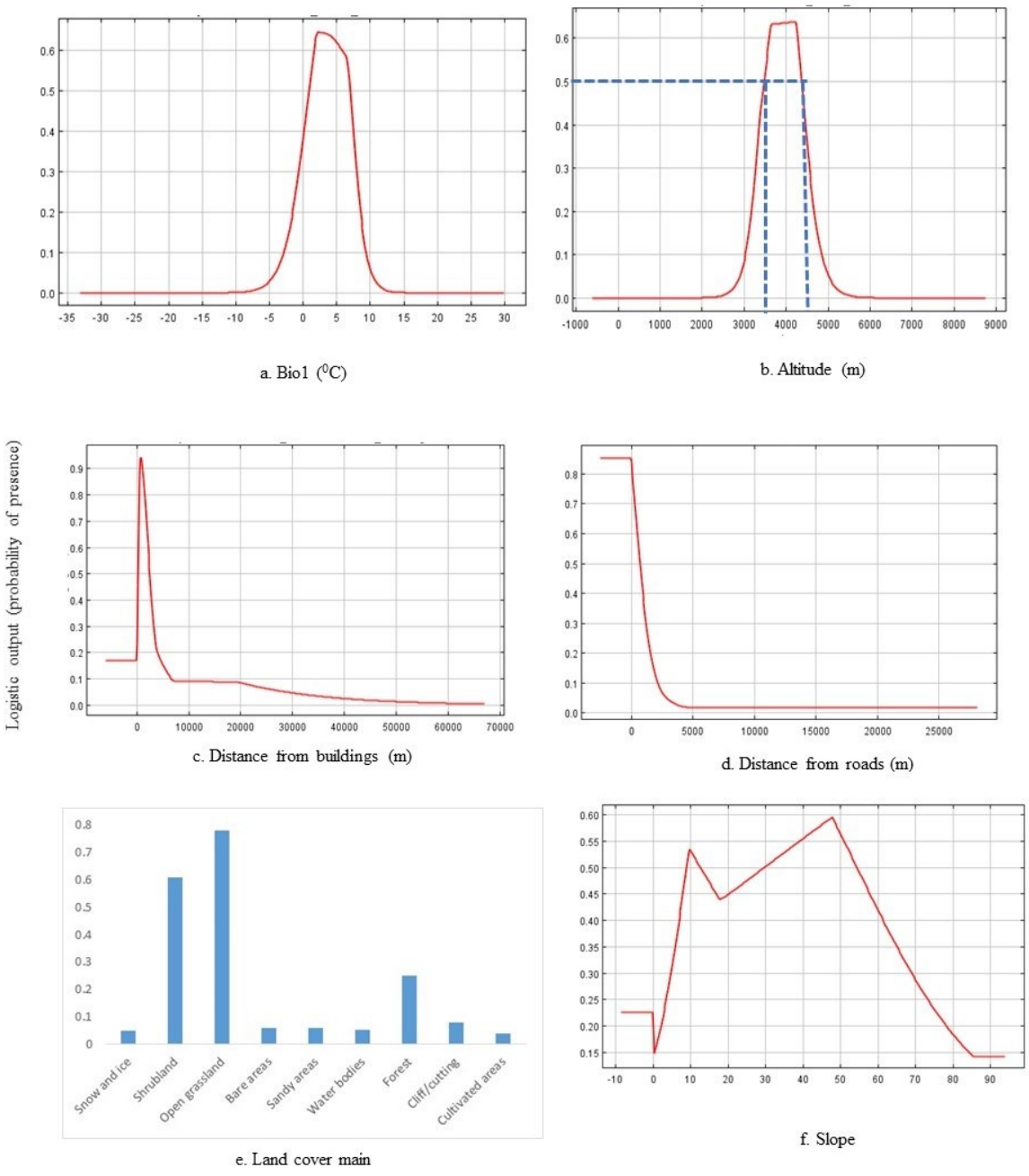

Fig. 6.11 Response curve of selected variables for snow leopard habitat suitability in Nepal

6.5 Discussion

Our results revealed that annual mean temperature and altitude were the two main factors influencing snow leopard habitat suitability in Nepal using the MaxEnt model. As these two factors are strongly correlated, one can conclude that the main factor driving the presence of snow leopard is just one of them – biologically more meaningful is altitude. The response curve of different predictor variables

of our study revealed that the probability of snow leopard presence in Nepal was highest in the altitude of around 4,000 m a.s.l. with a relatively cold and dry climate, shrubs, rocks and open grassland.

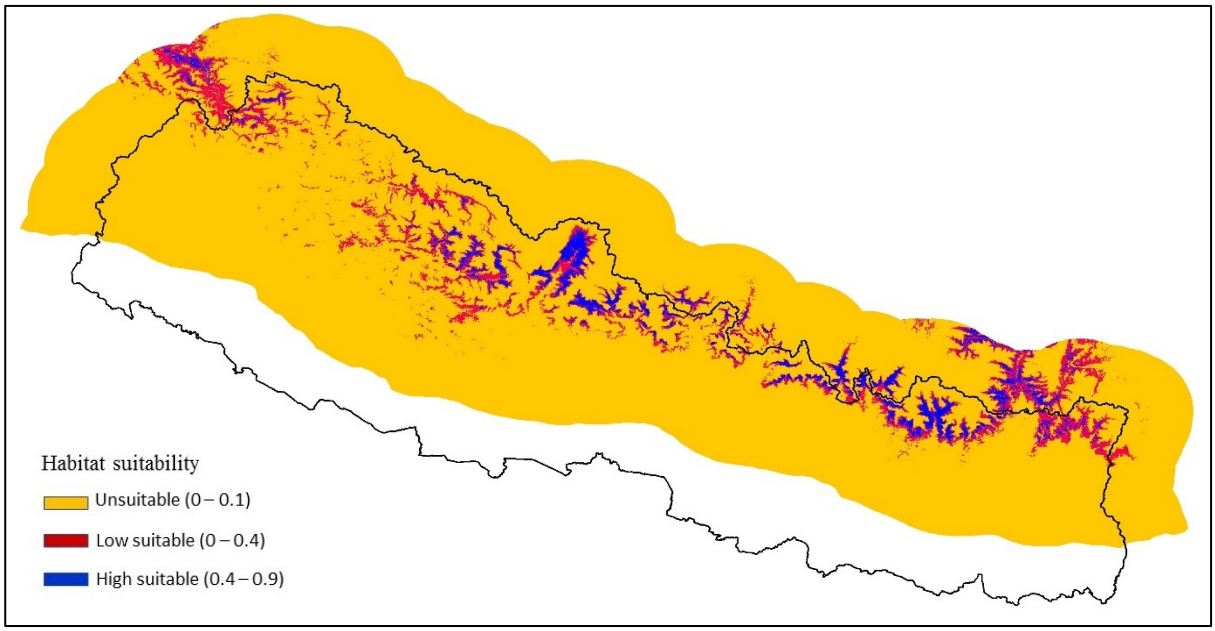

Fig. 6.12 Map of Nepal showing the distribution of suitable habitat for snow leopard predicted by the model. The black line is Nepalese border. The map overlaid does not exactly coincide with area of Nepal, as also adjacent parts of Tibet were considered. Different colours indicate the probability of occurrence of snow leopard (see inset). The most suitable areas are in blue

Some other factors also seem to play a role, as they were important in at least one of the analyses. These were: distance from road, distance from buildings, land cover main associated with grassland and shrubland, precipitation of driest month and precipitation of warmest quarter. What are the conclusions of other studies regarding the factors influencing snow leopard habitat suitability in Nepal?

Using the ***MaxEnt model***, Bai et al. (2018) suggested five main factors (driest quarter, ruggedness, altitude, maximum temperature of the warmest month, and annual mean temperature) in the Qomolangma National Nature Reserve, Li et al. (2013) reported two main factors: annual mean temperature and ruggedness in Sanjiangyuan National Nature Reserve, Watts et al. (2019) reported two main factors : altitude and ruggedness and Aryal et al. (2016) reported only one factor: annual mean temperature. Atzeni et al. (2020) reported landscape attribute associated with snow leopard habitat requirements, consisting of a positive association with annual mean temperature, medium/large extent of grassy and herbaceous vegetation on ridges and uplands and aggregated low-contrast landscapes. These studies showed that climatic factors (e.g., annual mean temperature) and altitude were the main determinants, similarly to our study. A contrasting result, however, was found

for ruggedness, the presence of which is ecologically considered as profitable for escape and shelter, by Jackson (1996).

Based on the ***resource selection function*** (statistical methods), ruggedness, bases of cliffs and stream beds in Xinjiang China (Xu et al. 2012), terrain and distance to trail and settlement in Sagarmatha National Park (Wolf and Ale 2009), cliffs, grassland and shrubland at high elevations (3,000–5,000 m a.s.l.) in Upper Mustang, Nepal (Aryal et al. 2014), and distribution of prey resources in Spiti Valley, India (Sharma et al. 2015) were suggested to be the main factors determining snow leopard spatial distribution. Likewise, altitude had the strongest influence on site use whereas the influence of presence of prey and distance to roads was relatively weak (Alexander et al. 2016).

Using ***radio-collar monitoring***, McCarthy et al. (2005) reported steep and rugged terrain, high use of areas rich in ungulate prey, and affinity for habitat edges were the main predictors to determine the snow leopard distribution.

The most often suggested factor determining snow leopard distribution in the studies above was annual mean temperature, similarly to our results. A broad array of both abiotic (altitude, terrain rugged and annual mean temperature) and biotic factors (prey availability and human activity) was also suggested, which indicates that these factors may differ between areas. It was evident that temperature factors were strongly affecting snow leopard habitat selection in most of the studied regions, which is suggesting that climate change might affect snow leopard distribution in the future (Li et al. 2016). The disturbance by humans may further affect the living conditions of snow leopard, as with human expansion the lower altitudinal limit of snow leopard distribution may be shifted upwards in the future, which will further restrict the size of its suitable habitat.

With regard to human activity, based on response curve, the presence of snow leopard sign is usually positively related to distance from trails and settlements, indicating decreased activity in the proximity of human activity (Wolf and Ale 2009). Unlike the present study, Atzeni et a. (2020) found higher occurrence patterns of snow leopard at low density of roads and human settlements. The rarity of sightings of snow leopards in the wild suggests that these animals tend to avoid humans (Jackson 1996; Schaller 1977; Wolf and Ale 2009). This would match the recorded effects of human activity on habitat use by other large predators such as grizzly bears, wolves, and tigers (Ciarniello et al. 2007; Johnson et al. 2006; Linkie et al. 2006; Whittington et al. 2005; Wolf and Ale 2009). Generally, predators avoid large or frequented roads and trails, especially in areas where hunting or harassment is common (James and Stuart-Smith 2000; Kaartinen et al. 2005; Linkie et al. 2006; Whittington et al. 2005; Wolf and Ale 2009). Thus, humans may be a substantial determinant of where snow leopards are active. This is in accord with the results of our study.

To conclude: the habitat suitability models indicate that the main danger for snow leopard survival may be climate warming and human expansion. Both these phenomena will push the lower

limit of its distribution upwards to higher elevations, which will entail two negative effects. One is a decline of the size of snow leopard suitable habitat, which may result to further decline of its metapopulation size and of the size of each of the local populations, together with all negative effects typical for small populations. The other one is decline in migration rate between individual populations. This may be caused by disappearance of stepping-stones between individual patches, where snow leopard is present, as these stepping-stones will "drown" when the lower limit of the altitude suitable for its distribution will be pushed upwards.

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Paper II

Non-Invasive Genetic Sampling of Snow Leopards and other mammalian predators in the Annapurna and Sagarmatha regions of Nepal

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Chapter 7: Shrestha B, Sherchan AM, Joshi J, Karmacharya D, Kindlmann P (accepted) Non-Invasive genetic population survey of snow leopard and its co-predators in Annapurna and Sagarmatha region of Nepal. In: Kindlmann P. (ed.) Population dynamics of snow leopard, Springer, Dordrecht, in press.

ABSTRACT

Snow leopard is threatened particularly by habitat loss, reduction in the availability of prey, conflict with herders and poaching in connection with traditional Asian medicine. Therefore, an effective conservation strategy for snow leopard is needed. For this, however, we need estimates of its abundance and map its spatial distribution. The problem is that because of the rugged, practically inaccessible terrain inhabited by snow leopards, its elusive nature and low population densities, there is very little information on its distribution and population status. Thus, in order to supplement conventional techniques like surveying for signs of its presence a more effective method is needed to ensure the long-term survival of this endangered felid. Non-invasive genetic sampling and molecular scatology are emerging and promising scientific techniques for sampling mammals. Their benefit is that the target species never has to be directly observed or handled, as the most commonly sampled are their hairs and scats. However, the collecting of scat samples of a focal species in the field is subject to a high degree of misidentification as they can often belong to other species. Therefore, genetic analysis of DNA extracted from scat samples must be used. Here we study the distribution and population size of snow leopards based on a genetic analysis of scat.

7.1 Introduction

Snow leopard *(Panthera uncia)* is an umbrella species for the Himalayas and vulnerable throughout its range, which includes 12 states in Asia (McCarthy et al. 2017). In Nepal, they are threatened particularly as a result of habitat loss, low availability of prey, conflict with herders and poaching in connection with their use in traditional Asian medicine. Although snow leopards in Nepal are protected under Schedule 1 of the National Parks and Wildlife Conservation Act, 1973 and a priority species for conservation, their numbers are declining dramatically. Thus, there is an urgent need to develop an effective conservation strategy in order to prevent the extinction of snow leopards (DNPWC 2017). Other carnivores such as wolf (*Canis lupus*), Eurasian lynx (*Lynx lynx*), red fox (*Vulpes vulpes*) and golden jackal (*Canis aureus*) co-occur across the snow leopard's range. Common leopard (*Panthera pardus*) also partly overlaps the snow leopard's range near the timber line. Understanding the interactions between these sympatric species is crucial for understanding snow

leopard population dynamics. Before any effective conservation plans can be developed and implemented, it is important to obtain reasonable estimates of the current abundance and distribution of snow leopards and its co-predators, and produce maps of its spatial distribution.

However, because of the rugged and practically inaccessible terrain inhabited by snow leopards, its elusive nature and low population density, very little information is available on its distribution and population status (Jackson et al. 2006). Thus, an effective, scientific and standardized method is needed to supplement conventional techniques like surveys of signs of their presence, and so ensure the long-term survival of this endangered felid. Promising scientific techniques such as non-invasive genetic sampling and molecular scatology may yield realistic population estimates of snow leopards when complemented with information obtained using conventional techniques (Waits and Paetkau 2005; Waits et al. 2007; Broquet et al. 2007; Janecka et al. 2008). The benefit of noninvasive sampling is that the target species never has to be directly observed or handled, as the most common samples used in this case are hairs and scats (Caragiulo et al. 2016). However, when collecting scat of a focal species like snow leopard in the field it is likely it will be misidentified as it that of another species. Therefore, genetic analysis of DNA, extracted from scat, is needed to accurately identify whether it is that of snow leopard or its co-predators. Here we study the distribution and population size of snow leopards along with that of other co-occurring carnivores (wolf, common leopard, red fox and golden jackal) based on a genetic analysis of samples of scat.

7.2 Study Areas

The areas studied are in three important snow leopard areas in Nepal: (i) Lower Mustang (LM), (ii) Upper Manang (UM), both of which are in the Annapurna Conservation Area (ACA), and (iii) Sagarmatha National Park (SNP). Details of these areas are presented in chapter 9.

7.3 Methods

7.3.1 Scat Sampling

From 2014 to 2016, we established transects covering a total linear distance of 139.3 km (102 transects, mean length 776 m, range 400–1,200 m, SE = 34.5) in both summer and winter at the three sites studied. These transects were of the type used by the Snow Leopard Information Management System (SLIMS; Jackson and Hunter 1996). With the aid of 1:50,000 topographic maps, we established transects along land forms such as ridgelines, narrow valleys, trails and cliff-edges, where

snow leopards are likely to walk and leave signs (Fox et al. 1991; Mallon 1991; Jackson 1996). Along these transects and by additional random collections during 2011–2013, we collected 268 putative snow leopard samples (261 samples of faeces, 6 samples of hairs and one urine sample). For each scat found, a small portion was placed in a 15 ml plastic tube with silica desiccant and subsequently subjected to a DNA analysis (Janecka et al. 2008).

7.3.2 Extraction of DNA used in the identification of Snow Leopard

DNA from faeces and hair was extracted using a Qiagen QIAamp DNA Mini Stool Kit (Qiagen 2014). For identifying snow leopards, a ~148 bp segment of mitochondrial cytochrome b was PCR-amplified using the primers CYTB-SCT-PUN-R' and CYTB-SCT-PUN-F' (Farrel et al. 2000). The targeted region is unique for snow leopards (Fig. 7.1) (Janecka et al. 2008).

The 7 µl of PCR reaction mixture included 1.4 µl 5X PCR Buffer, 0.035 µl 5000 Units of Taq Polymerase, 0.21 µl of 10 µM forward and reverse primers, 0.14 µl of 10 mM dNTPs and distilled water (3 µl) to which 2 µl DNA extract was added.

All PCRs were performed in duplicate for confirmation along with a negative and positive PCR controls. PCR conditions were as follows: Initial denaturation – 94 °C for 2 minutes followed by 95 °C for 30 seconds, annealing at 60 °C for 15 seconds followed by 68 °C for 1 minute (35 Cycles) and a final extension at 68 °C for 10 minutes. The PCR products were run on a 2% agarose gel, stained with Ethidium bromide and visualized under ultraviolet light (Janecka et al. 2008).

The PCR was performed in duplicate (2 of each sample), any result that was singlet positive was repeated for a third time in order to confirm the identification and duplicate positives were accepted as a positive identification and duplicate negatives that the DNA was that of another species.

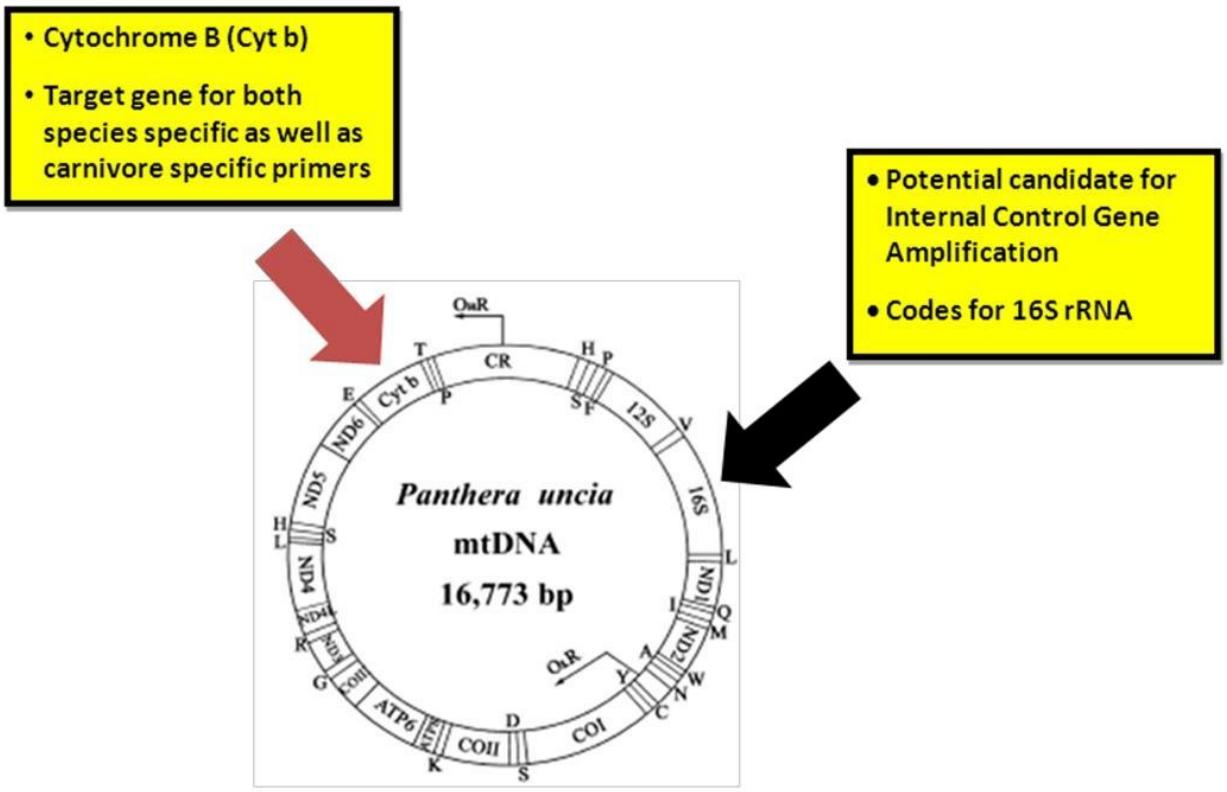

Fig. 7.1 Schematic diagram of the mitochondrial DNA of *Panthera uncia* depicting regions used for species and carnivore identification

7.3.3 ID PCR used to determine the sex of a Snow Leopard

The sex of verified snow leopards was determined by testing samples of their scat for the presence of a Y chromosome using primers that amplify an intron of the AMELY gene (i.e., a gene found only on the Y chromosome). The size of this fragment (Y chromosome) is about 200 bp (Janecka et al. 2008).

A volume of 7 µl of PCR reaction mixture containing 3.5 µl of Qiagen 2X Master Mix Buffer, 0.7 µl of 5X Q solution, 0.05 µl of primers (AMELY-F and AMELY-R) 20 µM, 0.7 µl of distilled water and 2 µl of extracted undiluted DNA was prepared. The PCR reaction was carried using the following thermocycling conditions: 95 °C for 15 minutes; followed by 45 cycles of 94 °C each for 15 seconds, 55 °C for 30 seconds and 72 °C for 1 minute with a final extension at 72 °C for 10 minutes. PCRs were run in triplicates along with a positive and negative control. The PCR products were run on a 2% agarose gel, stained with ethidium bromide, and visualized under ultraviolet light. The PCRs were each triplicated (3 for each sample), three out of three male positives and two out of three male positives were considered positive. One of three male positives were repeated to re-confirm the sex. If the repeats were 1/3 for each triplicate, they were considered male if there were 6

positive replicates. Only 3 negatives were considered to indicate female. If only one out of six replicates indicated a Y chromosome, the samples were regarded as of unconfirmed sex.

7.3.4 Identification of individuals based on microsatellite loci

A set of 6 microsatellite loci located on 6 different chromosomes of snow leopard was targeted using the following six fluorescent dye tagged primers in two combinations. The six polymorphic microsatellite loci chosen were sufficient, using PID (Probability of Identity), for identifying individuals in a population with the same genotype. (Janecka et al. 2008) (Table 7.1).

The 7 µl volume of PCR reaction mixture contained 3.5 µl of Qiagen 2X Master Mix buffer, 0.7 µl of 5X Q solution, 2 µl of extracted DNA and 0.88 µl of 20 µM primers in the first combination and 0.78 µl of 20 µM primers and 0.02 µl of distilled water in the second combination. The multiple tubes method (Taberlet 1996) was used for the multiple PCR reactions for each sample, in this case with three replicates. PCR products from both combinations was further diluted to 1:50 and processed using an ABI 31 Analyzer (Table 7.2). All samples were processed in triplicate.

Table 7.1 The six microsatellite loci used for identifying snow leopards (Janecka et al. 2008, 2014; Rozhnov et al. 2011; Janecka et al. 2014)

Locus name	Repeat motif	Size range (bp)	Chromosome	Label
PUN1157	(AC)17	101–109	B3	Blue
PUN229	(GT)23	104–112	A1	Green
PUN124	(AC)22	90–100	A2	Black
PUN935	NA	110–120	D1	Blue
PUN894	(GT)17	110–118	C2	Green
PUN132	(GT)19	117–123	D3	Black

COMBINATION 1	SEQUENCE
PUN124-F	NEDCCATTCCCTCCCTGTCTGTA
PUN124-R	TGTCCTCAAACCATAGACAGTTTC
PUN229-F	VICAGACAAACTGACAAGCTTAGAGG
PUN229-R	TCATGTCTTTACATTCATTTCTTTTT
PUN1157-F	FAMGAGAGTGCAGTCAGCCAGGT
PUN1157-R	TGAAATTCAGCTGCTTCAACTC

COMBINATION 2	SEQUENCE
PUN132-F	NEDCGAAATGCAGTAATGTTAGTTTTACA
PUN132-R	CACGGGTTCGTCTCTTTTG
PUN894-F	VICCATGCCAGACTGCATTTGTT
PUN894-R	CCCACACATGACAATCCTGTT
PUN935-F	FAMGCTGCTGTGACCTTCTGTGA
PUN935-R	CAGTGTTCCTGGTTTGCTCA

Table 7.2 PCR profiles of the microsatellites used to identify individuals using a set of 6 primers in two different combinations, 1 and 2

Snow Leopard-Bikram Shrestha Individual ID uSAT-ANALYSIS_combination 1
1 Batch PCR (N=13)

SL-uSat PCR COM 1 Date: 15-May-16 JJ **Replica: 1,2,3**

Multiplex Mix	1x	n	Final Conc.	Unit
	1x	48	conc.	
Master Mix (2x)	3.5	168	1x	x
Q solution (5x)	0.7	33.6	0.5	x
PUN-124-F (20uM)	0.19	9.12	0.54	µM
PUN-124-R (20uM)	0.19	9.12	0.54	µM
PUN-229-F (20uM)	0.18	8.64	0.51	µM
PUN-229-R (20uM)	0.18	8.64	0.51	µM
PUN-1157-F (20uM)	0.07	3.36	0.2	µM
PUN-1157-R (20uM)	0.07	3.36	0.2	µM
dH20 from kit	0	0		
DNA	2			
Total volume	7.08	243.84		

Thermocycler: MJ Tetrad
Saved PCR Program: MSAT-WF_BS_SL
SNOW LEOPARD
Number of cycles: 45

	Temp. (°C)	Time
Initial denature	95	15 min
Denaturation	94	30 sec
Annealing	55	90 sec
Elongation	72	90 sec
Final elongation	72	10 min
Cool down	4	Hold

Snow Leopard-Bikram Shrestha Individual ID uSAT-ANALYSIS_combination 2
1 Batch PCR (N=13)

SL-uSat PCR COM 1 Date: 15-May-16 JJ **Replica: 1,2,3**

Multiplex Mix	1x	n	Final Conc.	Unit
	1x	48	conc.	
Master Mix (2x)	3.5	168	1x	x

Thermocycler: MJ Tetrad
Saved PCR Program: MSAT-WF_BS_SL
SNOW LEOPARD

Q solution (5x)	0.7	33.6	0.5	x	Number of cycles: 45		
						Temp. (°C)	Time
PUN-132-F (20uM)	0.22	10.56	0.54	µM			
PUN-132-R (20uM)	0.22	10.56	0.54	µM	Initial denature	95	15 min
PUN-894-F (20uM)	0.1	4.8	0.51	µM	Denaturation	94	30 sec
PUN-894-R (20uM)	0.1	4.8	0.51	µM	Annealing	55	90 sec
PUN-935-F (20uM)	0.07	3.36	0.2	µM	Elongation	72	90 sec
PUN-935-R (20uM)	0.07	3.36	0.2	µM	Final elongation	72	10 min
dH20 from kit	0.02	0.96			Cool down	4	Hold
DNA	2						
Total volume	7	240					

After processing the 6-microsatellite based PCR products using capillary electrophoresis and an ABI 31 Analyzer, the labelled fragments separated on the basis of their size were retrieved in .fsa files. All the data in these .fsa files were analyzed and used to assign them to particular alleles. Allele calling was done using GeneMarker V1.85 (http://www.softgenetics.com) and then consensus genotypes were reconstructed (for details see Benesova 2018).

7.3.5 Identification of Common Leopard

Samples from scats that were negative for snow leopard were further tested using PCR to quickly identify whether they came from common leopard scats. For this, common leopard was identified by targeting the NADH region with the primer set NADH4F/R as mentioned in Mondol et al. (2009), which amplifies a segment of 130 bp. The primer set included as follows:

NADH4 F - 5’ TRATAGCTGCYTGATGAC-3’
NADH4 R- 5’ GTTTGTGCCTATAAGGAC-3’ (Mondol et al. 2009)

The 7 µl PCR reaction mixture contained 3.5 µl of 2X Qiagen Master mix, 0.7 µl of 5X Q solution, 0.20 µl of 20 pmol/µl of each primer, 0.5 µl of distilled water to which 2 µl of extracted undiluted DNA was added. PCR thermo-cycling condition: 95 °C for 15 minutes followed by 50 cycles each of 94 °C for 30 seconds, 50 °C for 30 seconds and 72 °C for 30 seconds and a final extension of 72 °C for 10 minutes. Five µl of the amplified PCR products, with incorporated positive controls, were visualized in 2% agarose gel by staining with 6.5 µl EtBr (Mondol et. al 2009).

7.3.6 Identification of the sex of common leopards

DNA samples positive for common leopard were further screened to determine the sex by amplifying the Amelogenin area on sex chromosomes using specific PCR primers (AMEL-F and AMEL-R). The sex of the verified common leopard scat was determined by testing for the presence of the Y chromosome (196 bp) on which there is a 20 bp deletion not present on the X chromosome (214 bp). The primers used were as follows:

AMEL-F 5'- CGAGGTAATTTTTCTGTTTACT-3'
AMEL-R 5'- GAAACTGAGTCAGAGAGGC-3'

The 7 µl PCR reaction mixture contained 3.5 µl of 2X Qiagen Master mix, 0.7 µl of 5X Q solution, 0.05 µl of 20 pmol/µl of each primer and 0.7 µl of distilled water to which 2 µl of extracted undiluted DNA. PCR thermo-cycling condition: 95 °C for 15 minutes followed by 45 cycles each at 94 °C for 15 seconds, 55 °C for 30 seconds and 72 °C for 1 minutes, with a final extension at 72 °C for 10 minutes. The PCR products, with incorporated positive controls, were subjected to electrophoresis and visualized in 3% agarose gel.

7.3.7 Identification of other carnivores

Carnivore specific PCR was performed on samples that were not identified as either snow leopard or common leopard. For the carnivore specific PCR, a specific PCR primer set (CYTB-SCT F and CYTB-SCT R) was used, which targets a 150 bp region of cytochrome b (Farrell et al. 2000). The primer set used was as follows:
CYTB-SCT-F: 5' AAACTGCAGCCCCTCAGAATGATATTTGTCCTCA 3'
CYTB-SCT-R: 5' TATTCTTTATCTGCCTATACATRCACG 3'

The PCR reaction mixture of total volume 25 µl contained 5 µl of 5X One Taq Standard Reaction Buffer, 0.50 µl of 10 mM dNTPs, 0.125 µl of 5000U One Taq DNA polymerase, 0.75 µl of each primer and 15.9 µl of distilled water to which 2.0 µl of extracted undiluted DNA. The PCR thermo-cycling conditions: 94 °C for 30 seconds followed by 45 cycles each at 94 °C for 30 seconds, 55 °C for 30 seconds and 72 °C for 1 minute with final extension step at 68 °C for 10 minutes. The PCR products, along with an incorporated carnivore positive control, were visualized in 2% agarose gel. The carnivore positive PCR amplicons were sequenced on an ABI 310 machine using the forward primer (CYTB-SCT- F); DNA sequences were BLAST searched in the NCBI database to identify the species of carnivore. Species identification of samples using BLAST results were based on cytochrome b DNA sequence data of 100 bp or more, with a maximum identity of 95% or higher and query coverage of more than 95%.

7.4 Results

7.4.1 PCR identification of snow leopards

Out of the total of 268 samples of scat, hairs and urine obtained in three areas, 128 (48%) were identified as snow leopard (124 scat and 4 hair samples) based on species specific mitochondrial DNA (Fig. 7.2).

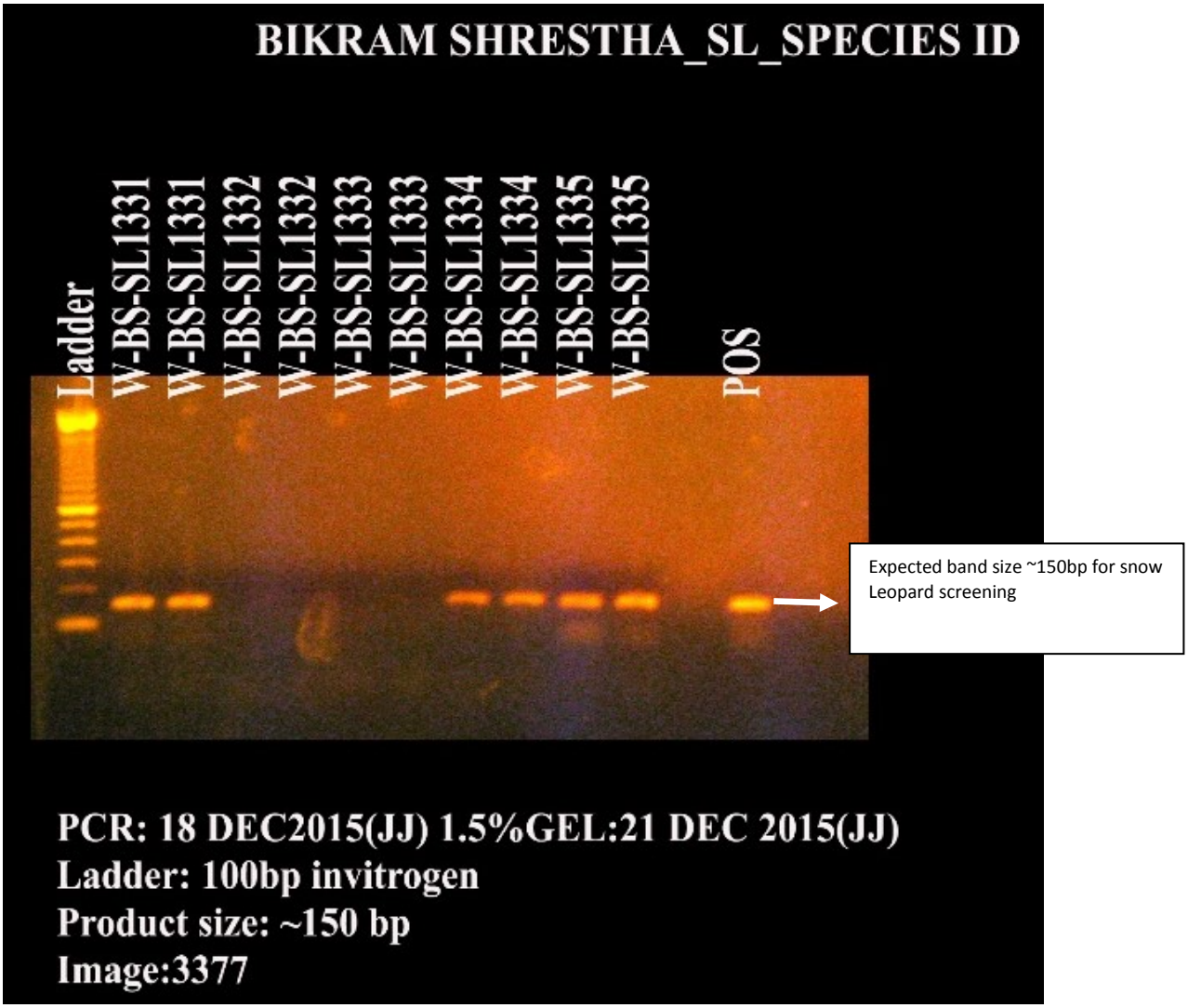

Fig. 7.2 Identification of snow leopards using gel electrophoresis and targeting the specific cytochrome b region (~150 bp) of *P. uncia*. Samples were duplicated and the positive control appeared in the expected region on the gel

7.4.2 PCR identification of the sex of snow leopards

Out of the samples positive for snow leopards that were PCR amplified and used for identifying the sex (Fig. 7.3), 40 were for males and 61 for females, and 10 did not identify the sex.

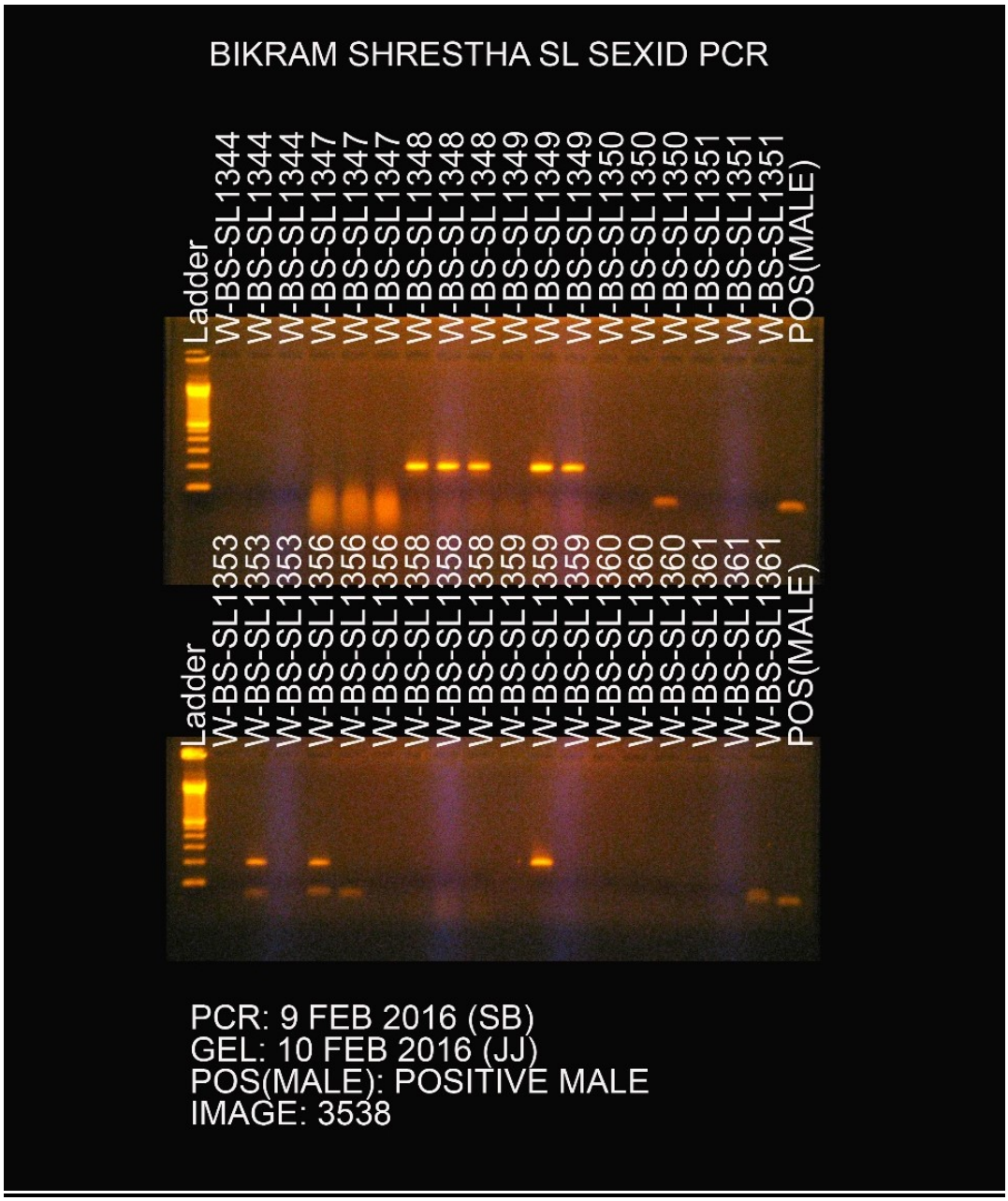

Fig. 7.3 Identifying the sex of the snow leopards by targeting the AMELY gene of ~200 bp specific for males, which was revealed by a band in the appropriate position when subjected to electrophoresis run on a 2% agarose gel, none of the samples from females were positive

7.4.3 Processing of microsatellite chromatograms

Microsatellite loci were not identified in twenty samples from snow leopards, therefore, these samples were not included in the following analyses. The number of samples in which microsatellites of snow leopards were identified was 108. After data quality filtering, 63 microsatellite genotypes were obtained, corresponding to 22 individuals according to the identity analysis (Table 7.3). All loci were free of errors due to large allele drop out and stuttering, but two loci were suspect due to the presence of null alleles with estimated percentages of 20.27% and 14.45%, respectively. These two loci had a higher proportion of homozygotes than expected based on the allele frequency.

Of the 22 individuals, eight, ten and five were, respectively, recorded in LM, UM and SNP having only one individual (SL3) was recorded in LM and UM and 14 (63%) were males and 8 (37%) females (Table 7.3). Of the 14 males identified, six were recorded in LM, five in UM and three in SNP. And of the eight females, two were recorded in LM, five in UM and two in SNP.

Table 7.3 The sex and numbers of individual snow leopards identified based on microsatellite DNA sequencing. P = Positive snow leopard, PM = Positive male snow leopard, PF = Positive female snow leopard, Spr = Spring; Win = Winter. Data on individuals 1–22 are based on the results of Benesova (2018)

Area/Year	Samples	P	PM	PF	Individuals (Male)	Individuals (Female)	Total
Lower Mustang							
2011	17	13	1	9	1 (LM-SL1)	2 (LM-SL2, LM-SL3)	3
2013	3	2	1	0	1 (LM-SL1)		1
2014	23	12	5	1	4 (LM-SL4, LM-SL5, LM-SL6, LM-SL1)	2 (LM-SL3) , LM-SL2)	6
2016	31	17	8	9	2 (LM-SL7, LM-SL8)	2 (LM-SL3, LM-SL2)	4
TOTAL	74	44	15	19	6	2	8
Upper Manang							
2012	4	4	3	1	2 (UM-SL9, UM-SL10)	1 (UM-SL11)	3
2014	47	12	4	7	2 (UM-SL9, UM-SL10)	3 (UM-SL11), UM-SL12, UMSL13	5
2016	39	24	13	11	3 (UM-SL9, UM-SL14, UM-SL15, UM-SL16)	4 (UMSL13, UM-SL3, UM-SL11, UM-SL17)	7
TOTAL	90	40	20	19	5	5	10
Sagarmatha National Park							
Spr, 2015	66	31	12	15	2 (SNP-SL18, SNP-SL19)	2 (SNP-SL20, SNP-SL21)	4
Win, 2015	38	13	3	8	2 (SNP-SL18, SNP-SL22)	2 (SNP-SL20, SNP-SL21)	4
TOTAL	104	44	15	23	3	2	5
Overall	268	128	50	61	14	8	22

7.4.4 PCR identification of common leopards and their sex

Seventy nine of 140 samples that were negative for snow leopard amplified positive for NADH-4 (Fig. 7.4). Of these, 39 were female, 23 male and 17 of unidentified sex.

7.4.5 Screening of the samples for evidence of the presence of other carnivores

Of 61 samples that were negative for snow leopard and common leopard, 47 amplified positive using CYTB SCT F/R primers, which indicates the presence of other carnivores in the band size ~150 bp (Fig. 7.5). These carnivore ID positives were sequenced and the species identified using BLAST. Of these, 16 were Himalayan wolf (*Canis lupus*), 6 golden jackal (*Canis aureus*) and 23 red fox (*Vulpes vulpes*) (Table 7.4) and the remainder were not identified.

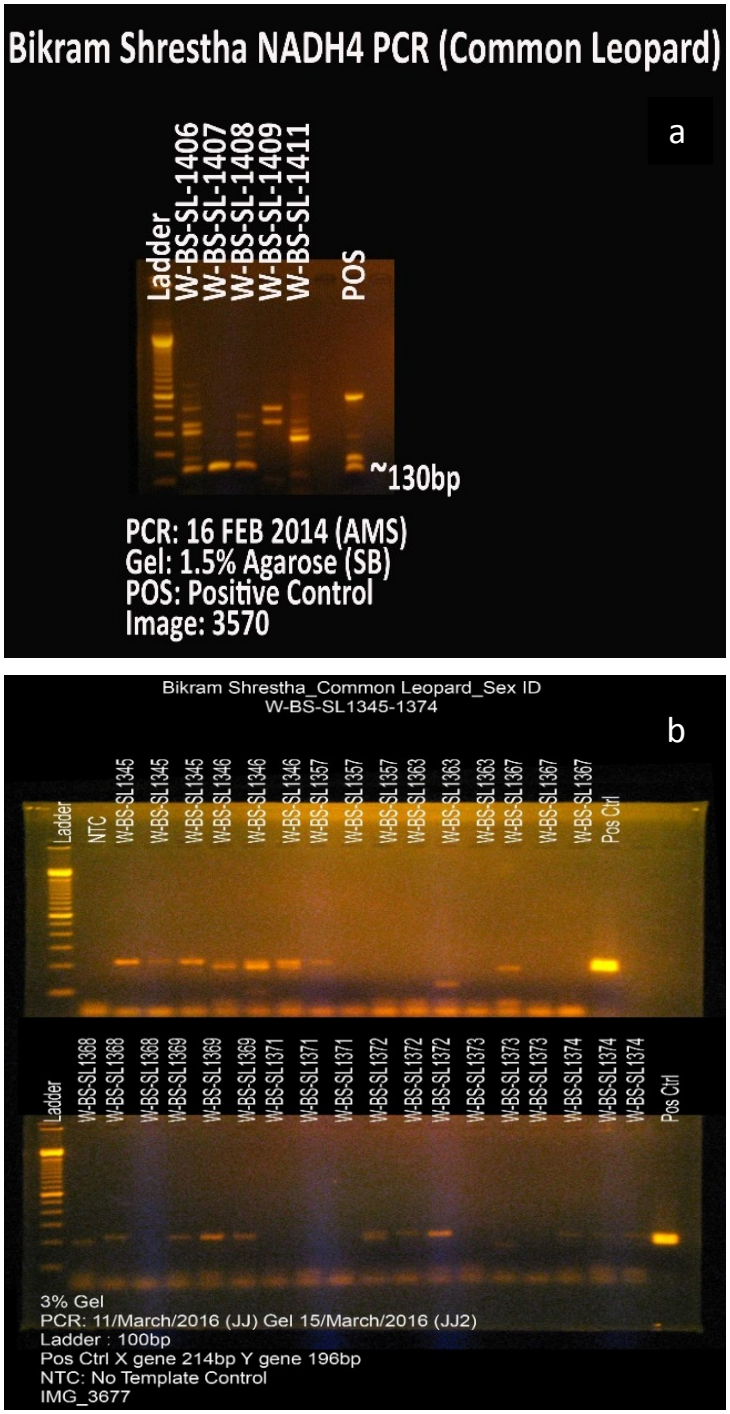

Fig. 7.4 a) PCR identification of common leopards using NADH4 F/R primers. Amplified bands are expected at 130 bp. b) The sex of the common leopards was identified using electrophoresis of amplified AMEL genes, of which the band size for males is ~ 196 bp and females ~214 bp

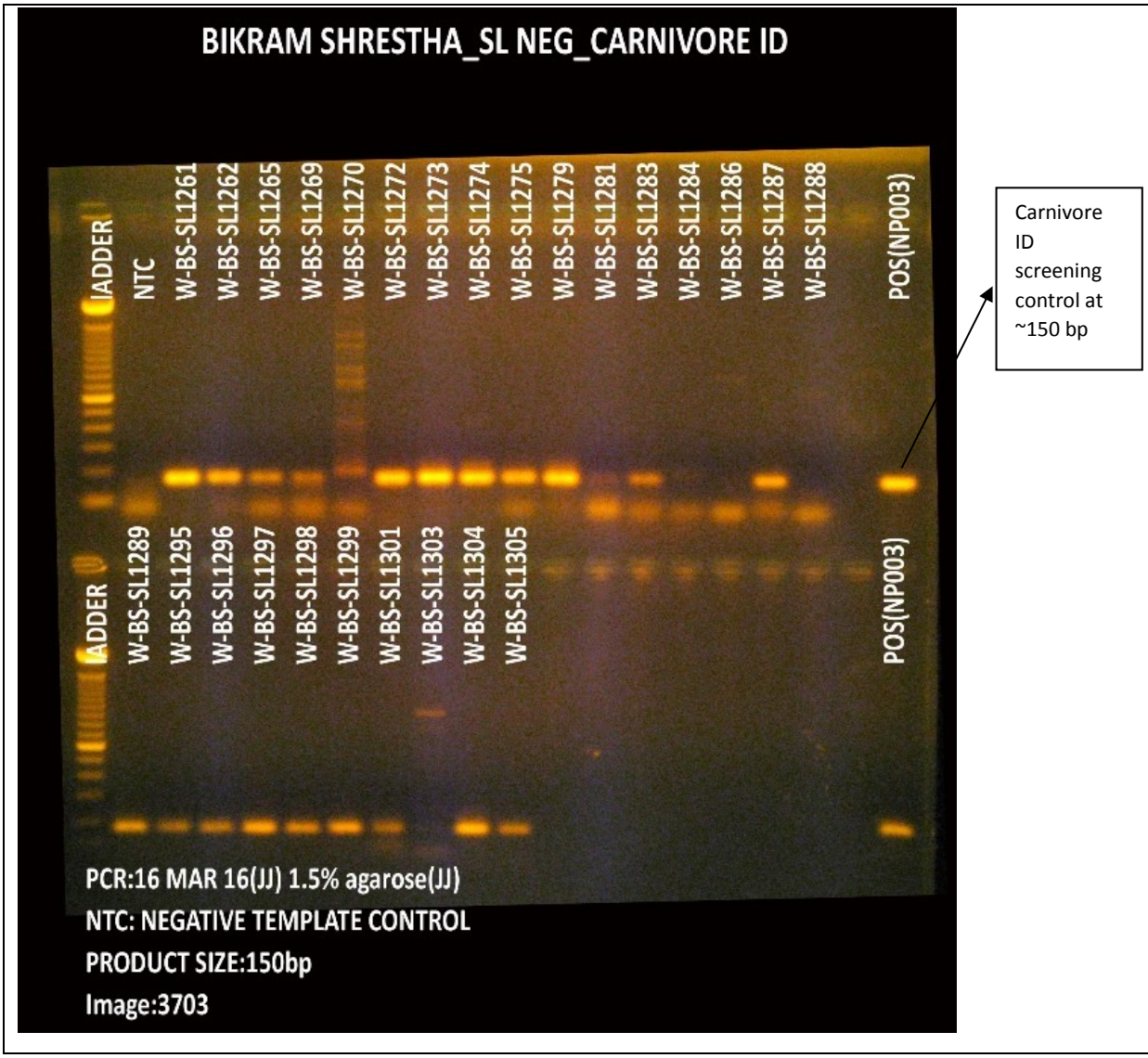

Fig. 7.5 Screening of snow leopard and common leopard negatives for general Carnivores (~150 bp) using electrophoresis on a 2% agarose gel stained with Ethidium Bromide

Table 7.4 NCBI BLAST results of the targeted amplification of the cytochrome b region of mtDNA in snow leopard and common leopard negative samples

Samples	PCR DNA length (bp)	Identified gene and Species	Match %	Query %
6	100, 160, 165	*Canis aureus* haplotype CytbBlg01 cytochrome b (cytb) gene, *Canis aureus* isolate CAU_Kenya_2 mitochondrion	62–99	91–95
16	89, 90, 92, 103, 150, 160, 165	*Canis lupus lupaster* cytochrome b (CYTB) gene, *Canis lupus* isolate Alaska 28	60–100	90–98
23	60, 110, 130, 140, 150, 155, 160, 165	*Vulpes vulpes* haplotype U52 cytochrome b (cytb) gene, *Vulpes vulpes* mitochondrion	65–100	86–96

7.5 Discussion

Of the scats analyzed in this study 50% were produced by the target species (snow leopard) and 50% by four other carnivores, namely common leopard, Himalayan wolf, golden jackal and red fox, which is in accordance with the findings of previous research (e.g., Janecka et al. 2008; Anwar et al. 2011; Janecka et al. 2011; Karmacharya et al. 2011; Shehzad et al. 2012; Jumabay-Uulu et al. 2013). It was thought that all the wolves in the Annapurna Conservation Area were killed by local herders four decades ago in retaliation for killing livestock. The genotyped scats and camera trap images (see in Chapter 5), showed that Himalayan wolves had recolonized the area. In addition to the snow leopard-human interaction in the area, the wolves are likely to have increased the conflict. Detailed studies of the interaction between snow leopards and wolves are needed as this information is likely to help mitigate their effects on humans.

In three of the areas studied, the abundance of snow leopards recorded using genetic analysis was higher than that recorded using camera traps (19 individuals) (see Chapter 5). Similar results using these techniques are reported for snow leopard in Mongolia (Janecka et al. 2011). The difference is because the non-invasive genetic survey could not be used to determine age and exclude sub adults, the limited spatial distribution of sampling points as a consequence of collecting scat along linear transects and deposition of scats by several snow leopards at the same site.

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Paper III

Population Dynamics of Snow Leopard and its Prey Species in Annapurna and Everest Regions of Nepal

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5.1 Introduction

This chapter is focusing on trends in changes of population sizes of snow leopard and its prey in Nepal.

The SNP is one of the most pressing regions regarding snow leopard survival. Because of adequate protection measures, snow leopards have returned to the SNP after forty years gap of its occurrence (Shrestha 2004 and 2006; Ale and Boesi 2005). For assuring its survival there, a number of questions raises. In this chapter we will address following ones: (i) What are the expected trends in population size and demographic structure of both snow leopard and its prey – Himalayan tahr – in the Sagarmatha region? (ii) Is snow leopard population in the SNP viable over a long run? (iii) Will the existing Himalayan tahr population be able to provide enough food for the snow leopard population of sufficient size to survive? (iv) What are the answers to these questions, when other areas of Nepal are considered?

Data on population size of Himalayan tahr from 1989 and 2010 in SNP have already been presented (Shrestha 2004 and 2006; Ale 2007; Lovari et al. 2009; Ferretti et al. 2014). However, the variability in mortality rate of different sex age classes of Himalayan tahr, and relation between snow leopard density and reproductive rate of Himalayan tahr are still missing. Better understanding of seasonal population dynamics is vital for predicting the impact of snow leopard density on Himalayan tahr or relationship between predator and prey.

Similarly, snow leopards have been reported in Lower Mustang and Upper Manang of ACA in several studies (Oli 1994; Ale et al. 2014) and in other areas of ACA (Aryal et al. 2014; Wegge et al. 2012), but a detailed knowledge of population trends in successive years is still lacking. We know virtually nothing about the perspectives of this species long-term survival. Long-term monitoring of snow leopard distribution patterns and demographic parameters, such as abundance, survival and recruitment, is fundamental for answering these questions. However, until now, such estimates have been based on indirect index methods such as sign encounter rates, because of snow leopard's highly elusive behaviour, rugged landscapes forming its territory, and because of limited resources and lack of manpower necessary for collection of such data. Therefore, data available on snow leopard biology

are scant, biased, and outdated and generally lack the scientific rigor (Fox et al. 1991; Jackson and Hunter 1996; Jackson et al. 2006).

Camera trapping is being increasingly deployed in monitoring of rare and shy wildlife (tiger: Karanth and Nichols 1998; Wegge et al. 2004; snow leopard: Jackson et al. 2006; McCarthy et al. 2008; Alexander 2016).

In this chapter, we fill the gaps outlined in the paragraphs above. Here we present our observations along with other published data on population abundance and trend of snow leopard and its main prey, Himalayan tahr and blue sheep. Additionally, population data of the main leopard prey (sex ratio, female to cub ratio) wherever these values were available are also presented. We perform basic analyses of these data and derive predictions useful for developing effective snow leopard management strategies.

5.2 Study Area

The study areas are located in three different snow leopard habitats of Nepal. Two of them are within the Annapurna Conservation Area (ACA): Lower Mustang (LM) and Upper Manang (UM), the third one is located in the Sagarmatha National Park (SNP). Details about these areas are given in Chapter 9 corresponding maps are shown in Figs. 9.1–9.4 and 9.6–9.7.

5.3 Methodology

5.3.1 Design of Monitoring Transects

We have established transects using the techniques of the Snow Leopard Information Management System (SLIMS; Jackson and Hunter 1996). With the help of 1:50,000 topographic maps, we laid transects along landforms, such as ridgelines, narrow valleys, trails and cliff-edges, where snow leopards are likely to traverse and leave signs (Fox et al. 1991; Mallon 1991; Jackson 1996). Our transects covered the most of the typical biotope of snow leopard. Transects were characterized by elevation, slope, distance to cliff and several topographic parameters. For their description see Table 5.1. However, the length of each transect was limited by local conditions: sometimes it was not possible to establish a sufficiently long transect to make ensure that each transect do not cross-obvious habitat boundaries. Long transects are more likely to cross habitat or landform boundaries, making the data more difficult to interpret and assign to a particular terrain type. Therefore, the lengths of transects unavoidably varied but no more than 1.2 km. We performed the transect monitoring during

the wet season (June - August) and during the dry season (September - March). The actual timing was also dependent on weather, however. In SNP we always walked the same transects, but in 2007 and 2009 a part of the transects (4 in 2007 and 7 in 2009) was not visited because leopard's signs were not recorded from those transects in 2006 at all and even local people did not claim their livestock kill by snow leopard from this area. SLIMS (Snow Leopard Information Management System, see Chapter 3) suggests it is a waste of time running transects in places avoided by snow leopard. In contrast, in 2015, some herders grazed their livestock in this area at high altitude in late autumn and winter (see chapter 8 in detail for livestock herding practice) and claimed that snow leopard had started to kill their livestock and therefore, we monitored again these transects. In UM and LM, the transects differed between years because much of areas were covered by thick snow in 2014 and we were limited by accessibility of study area. All parameters of these transects are specified in Table 5.2.

Table 5.1 Table of transects, main parameters and scrapes/km. Abbreviation and explanation of the parameters of this table are described in Table 5.2.

	Length [km]	SC/km	TFG	RUGG	HAB	CLD [m]	SLP [°]	ASP	Elevation [m]	Trail
LM, 2014 (Dry)										
LM14_01	0.8	5.0	RID	B	SC	500	45	SW	3351	HT
LM14_02	0.8	7.5	CLF	VB	SC	50	55	S	3761–3811	HT
LM14_03	0.8	7.5	RID	B	S	300	50	S	3957–4007	HT
LM14_04	0.8	6.3	CLF	B	S	100	40	S	3399–4125	HT
LM14_05	0.8	3.8	BOL	VB	G	200	35	NW	4252	HT
LM14_06	0.8	2.5	RID	B	G	100	50	SW	4480	HT
LM14_07	0.8	2.5	HS	ROL	OF	800	40	S	3705	HT
LM14_08	1	3.0	HS	ROL	S	800	40	S	4041	HT
LM14_09	0.8	3.7	RID	ROL	G	800	45	S	3618	HT
LM, 2016 (Wet)										
LM16_01	0.8	3.1	CLF	VB	SC	50	55	S	3761–3811	HT
LM16_02	1	5.0	CLF	VB	SC	50	100	S	4238–4394	HT
LM16_03	1	1.5	RID	ROL	OF	500	45	W	3934	HT
LM16_04	0.8	0.0	HS	ROL	G	100	55	N	4300	HT
LM16_05	1	0.5	HS	ROL	F	1000	45	N	3885	HT
LM16_06	0.8	0.0	BOL	VB	G	200	35	NW	4252	HT
LM16_07	0.8	0.0	RID	ROL	G	500	50	W	4450	HT
LM16_08	0.8	1.3	RID	VB	G	100	50	SW	4329	HT
UM, 2014 (Dry)										
UM14_01	0.8	3.1	RID	VB	S	200	55	S	3800–4047	HT
UM14_02	0.8	2.5	HS	VB	G	800	40	S	4609–4730	HT
UM14_03	0.8	6.3	HS	ROL	G	500	40	SE	4207–4350	HT

UM14_04	0.8	6.3	RID	ROL	S	800	45	N	3571	HT
UM14_05	0.8	2.8	RID	ROL	S	50	45	SE	3817	HT
UM14_06	1	6.0	RID	ROL	F	600	45	SE	3701–3809	HT
UM14_07	0.8	5.0	RID	ROL	S	200	50	SE	4232	HT
UM14_08	0.8	6.3	HS	ROL	S	500	40	SW	4207	HT
UM14_09	1.2	5.8	HS	ROL	S	500	35	SW	4265	HT
UM14_10	0.8	5.0	RID	ROL	G	800	40	SW	4350	HT
UM14_11	1	7.0	HS	B	S	400	40	SW	4287	HT
UM14_12	1	1.0	HS	ROL	S	520	50	SW	4300–4434	HT
UM14_13	1.2	6.7	RID	B	G	500	30	NE	4053	HT
UM14_14	0.8	5.0	RID	B	G	800	45	S	4347	HT
UM14_15	0.8	4.4	HS	ROL	S	600	40	SE	4324	HT
UM14_16	0.8	3.8	HS	ROL	S	800	40	S	4116	HT
UM14_17	0.8	7.5	HS	ROL	G	800	40	S	4461	HT
UM, 2016 (Wet)										
UM16_01	1	1.5	RID	ROL	G	1000	40	SE	4709	HT
UM16_02	0.8	0.6	RID	ROL	S	800	40	SE	4409	HT
UM16_03	0.8	2.5	HS	ROL	S	800	45	NE	4362	HT
UM16_04	0.8	5.0	HS	B	G	800	40	SE	4444	HT
UM16_05	1	20.0	RID	ROL	S	800	45	N	3571	HT
UM16_06	0.8	7.5	RID	VB	S	200	55	S	4200	HT
UM16_07	0.8	2.5	RID	VB	G	50	55	S	4730	NT
UM16_08	0.8	1.9	RID	B	G	500	45	SW	4574	HT
UM16_09	0.8	5.0	RID	VB	G	200	50	SE	4716	HT
UM16_10	0.8	1.3	HS	B	S	200	50	S	4543	HT
UM16_11	0.8	0.6	HS	B	G	1000	50	N	4601	HT
UM16_12	0.8	1.3	BOL	VB	S	100	50	N	4478	HT

TRANSECT	Length [km]	Sc/km, Dry 2006	Sc/km, Dry 2007	Sc/km, Dry 2009	Sc/km, Dry-spring 2015	Sc/km, Dry-autumn 2015	TFG	RUGG	HAB	CLD (m)	SLP [°]	ASP	Elevation (m)	Trail
SNP_01	0.85	1.78	1.18	0.00	0.39	1.18	HS	B	S	200	50	S	4293	MT
SNP_02	0.50	0.00	0.00	0.00	0.00	2.00	HS	B	G	200	45	S	4365	MT
SNP_03	0.80	0.00	–	–	0.83	1.25	HS	B	S	800	45	SW	4302	MT
SNP_04	0.30	0.00	–	–	3.33	0.00	CLF	VB	B	100	55	S	4844	NT
SNP_05	0.60	0.00	–	–	0.00	0.00	RID	B	G	1200	40	S	4531	HT
SNP_06	0.60	0.00	–	–	2.22	1.67	HS	B	S	500	45	SW	4534	HT
SNP_07	0.70	0.00	0.00	–	0.00	1.43	HS	B	G	800	30	W	4575	MT
SNP_08	1.00	2.50	1.50	0.83	5.50	0.00	HS	ROL	F	1000	40	S	3324	HT
SNP_09	0.40	5.00	7.50	0.00	0.00	0.00	RID	HS	S	1000	40	S	3691	HT
SNP_10	0.49	4.10	2.05	0.83	6.15	0.00	HS	B	S	800	45	S	3385	HT
SNP_11	0.40	3.75	5.00	0.83	0.00	0.00	HS	ROL	OF	800	40	s	3350	HT
SNP_12	1.00	2.00	1.00	–	0.00	0.00	HS	ROL	OF	1000	40	S	3500	MT
SNP_13	0.40	1.25	7.50	0.00	2.50	0.00	RID	B	F	800	50	S	3486	HT

SNP_14	0.20	10.00	7.50	1.25	1.00	5.00	HS	ROL	S	800	35	S	3868	HT
SNP_15	0.60	8.33	0.56	1.25	0.00	1.67	HS	ROL	OF	1000	45	SW	3636	MT
SNP_16	0.90	4.44	0.00	0.50	1.48	1.11	RID	B	S	100	45	S	3527	HT
SNP_17	0.65	6.15	1.54	0.63	2.46	1.54	HS	B	S	500	40	S	3977	MT
SNP_18	0.63	2.40	0.00	0.00	0.32	1.60	HS	B	S	400	45	S	3975	MT
SNP_19	0.40	0.00	0.00	–	0.00	0.00	HS	ROL	S	1000	40	S	3881	MT
SNP_20	0.50	10.00	1.00	1.25	0.40	2.00	HS	ROL	OF	800	45	S	4007	HT
SNP_21	0.50	1.00	0.00	–	2.00	2.00	RID	ROL	S	1000	45	S	3963	HT
SNP_22	0.90	0.00	0.56	0.00	0.00	2.22	HS	ROL	OF	1000	40	S	3861	HT
SNP_23	0.40	0.00	2.50	0.83	0.00	3.75	HS	ROL	F	800	35	S	3684	HT
SNP_24	0.41	0.00	–	0	–	–	HS	ROL	F	1000	35	S	3900	MT
SNP_25	0.50	3.00	1.00	0.00	0.00	0.00	RID	ROL	S	1000	35	SE	3699	NT
SNP_26	0.70	5.00	1.43	0.00	0.00	0.00	HS	ROL	S	1000	30	S	3552	HT
SNP_27	0.50	2.00	2.00	0.63	0.00	0.00	HS	ROL	S	1000	30	S	3550	HT
SNP_28	1.00	0.50	0.00	0.63	0.00	0.00	RID	ROL	S	800	40	E	3829	HT
SNP_29	1.00	0.50	0.00	0.00	0.00	0.00	HS	B	S	400	45	SW	3953	HT

Table 5.2 Description of transects parameters.

	parameter	**abbreviation/ name in table**	**explanation**
Study area	Lower Manang	LM	Name of study area
	Upper Manang	UM	
	Sagarmatha NP	SNP	
Year			Year of monitoring
Period		Dry / Wet	Dry or wet season of the year
Transect	code of transect	LM14_01 etc.	
Lenght [km]			Length of transect
Topography	Cliff	CLF	Terrain at site is very precipitous (slope > 500)
(TPG)	Ridge	RID	Narrow crest of land sloping down on either side
	Hill-slope	HS	Side or slope of a hill
	Scree/boulders	BOL	Accumulation of rocks and pebbles at base of a steep slope
Landform Ruggedness	Broken	BK	Terrain moderately broken by irregular slopes, rocky outcrops, and gullies
(RUGG)	Very broken	VB	Terrain heavily broken by rocky outcrops, ravines and gullies
	Rolling	ROL	Terrain has a relatively smooth land surface (e.g., rolling hills or alluvial fan)
Habitat	Barren	B	Less than 10% of the ground has vegetation cover
(HAB)	Grassland	G	Dominant vegetation is grassland

	Scrubland	SC	Dominant vegetation consists of bush (stem and leaves of a bush is usually almost touching the ground)
	Shrubland	S	Dominant vegetation consists of shrubs taller than bush
	Forest	F	Tree cover exceeds 30%
	Open Forest	OF	Dominated by open trees and grasslands
Cliff distance (CLD) [m]			Distance to the nearest cliff
Slope (SLP) [°]			Slope of transect
Aspect (ASP)	North	N	Predominant exposure of the transect to cardinal points
	East	E	
	South	S	
	West	W	
	South-west	SW	
	South-east	SE	
Trail	Herder trail	HT	Trail used only by herders, low intensity
	Main trail	MT	Main trail used by locals & tourists, high intensity
	Not trail	NT	Transect without regular trail
Elevation [m a.s.l.]			Elevation of the transect
Avg. Elevation [m a.s.l.]			Average elevation of the transect

We placed the transects covering the length 7–7.4 km/100 km^2 in LM, 9.5–14.3 km/100 km^2 in UM and 17.81 km/100 km^2 in SNP (Table 5.1). LM and UM of ACA have many broad ridges and hills flanking broad valleys — wide U-shaped valleys — where snow leopard travel routes were less well defined, thus making it difficult to locate their sign along transects. In contrast, SNP has sharp ridges and a deep gorge of river confluence — V-shaped rugged valleys — where snow leopard travel routes were better defined. In some analyses, we have also used the data collected by Ale (2007), but here we do not have exact descriptions of his individual transects.

5.3.2 Snow Leopard Abundance Estimated by Signs of its Presence

Snow leopard signs monitoring includes scrapes, scent (or spray), scats and pugmarks. Among them, encounter rate (sings/km) of scrapes marks are considered as the most reliable determinants for abundance indices. The monitoring of scats may be strongly biased, because genetic analyses showed that more than 50% of the scats collected, which looked like snow leopard scats, in fact belonged to other carnivores such as red fox etc. (Janecka et al. 2008; Shrestha et al. 2018). Similarly, footprints are hardly visible, when the trail is dry and hard, and difficult to detect footprints during heavy rainfall. Therefore, we analysed only scrape marks data for sign monitoring.

4.3.3 Snow Leopard Abundance Estimated by Camera Trapping

The relative abundances, population size and density of snow leopard were obtained by camera-trap surveys in the study areas during consecutive periods of sign survey in 2014 to 2016. For that, we used remotely triggered camera-traps (Bushnell HD camera, passive infrared detector Trophy Camera) (Fig. 5.1) along well-defined, narrow ridgelines or valleys, or immediately adjacent to frequently scent-sprayed rocks and scrapes. Study areas (areas surveyed) were divided into 4×4 km grids, which correspond to the average home range size of female snow leopards (Jackson 1996; Oli 1997). Depending on the complexity of the terrain, one or two camera stations were set in each grid cell, with one or two camera-traps per station, deployed at 2–3 m from the anticipated path of animals. These camera-traps were checked and batteries changed approximately every 7 to 10 days. We used 32–48 camera traps (depending on cameras availability) during two trapping sessions in each site (LM, UM, SNP), during dry (October-January) and wet seasons (April-August). The numbers of camera traps differed between the sites and seasons, but there were 4,567 trap-nights altogether.

Fig. 5.1 Researcher Bikram Shrestha installing a camera trap for monitoring in Manang, ACA.

We identified individual snow leopards by their unique spot patterns (Figs. 5.2, 5.3) and created photo-capture histories based on the time of individual capture. We categorized the photograph of snow leopard into full images (whole body visible) and partial images (visible is only part of the body). We used capture success rates (i.e., capture events/100 trap-nights) as a relative index of abundance (RAI). Pictures taken at time interval longer than one hour were considered as independent sightings (capture events). For pictures from interval shorter than one hour, we compared colour

patterns or other distinctive marks of the animals to determine, whether they were different or not. False images include blank photos whereas non-target images include photos of all other animals except snow leopard such as livestock, bird, wolf, red fox, human etc.

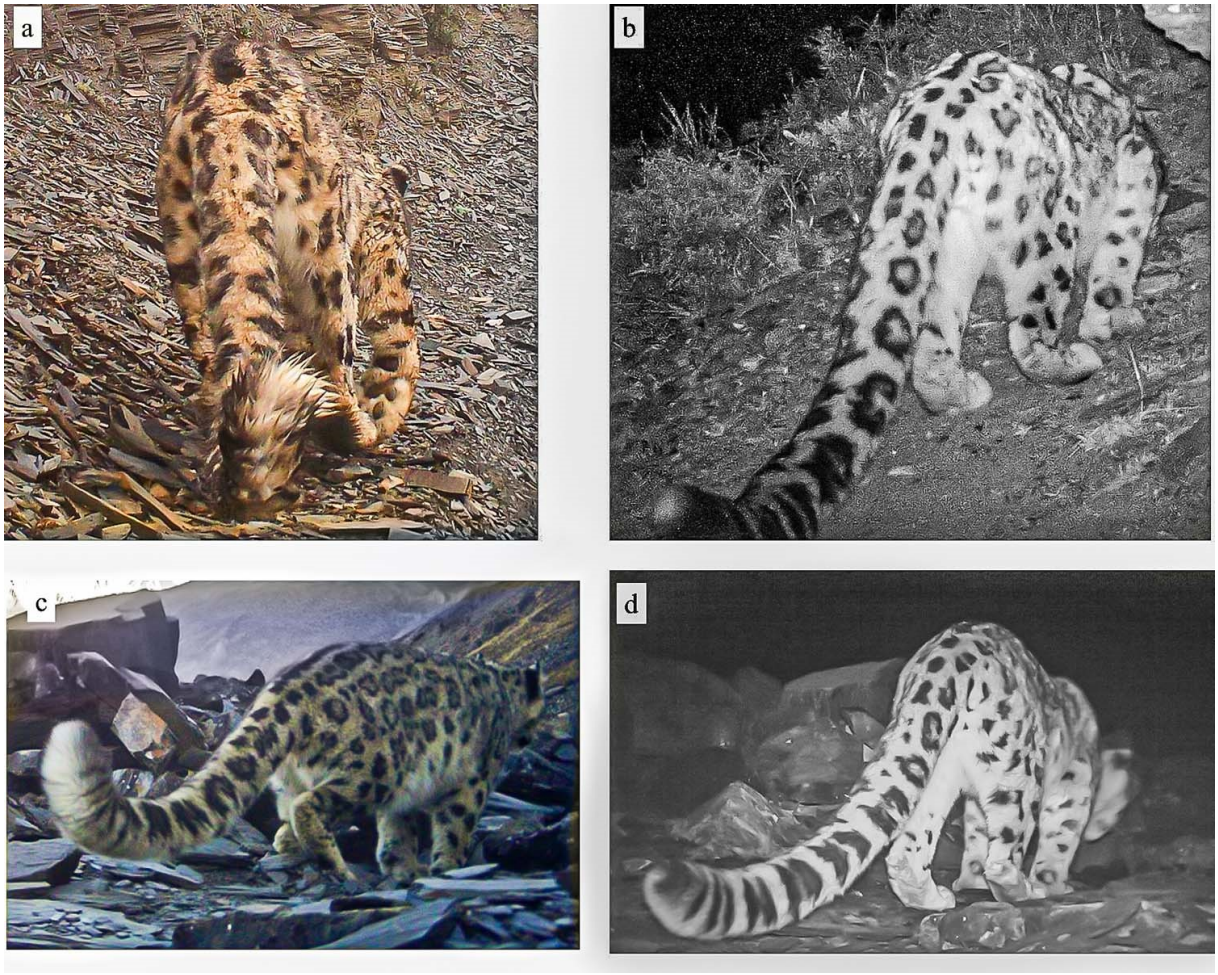


Fig. 5.2 Example of four different snow leopard individual identification through pelage pattern variation on dorsal surface of tail captured by camera trap in Lower Mustang and Upper Manang of Annapurna Conservation Area (2014–2016). (a) and (b), two different individuals from Upper Manang; (c) and (d), two different individuals captured at same place in Lower Mustang

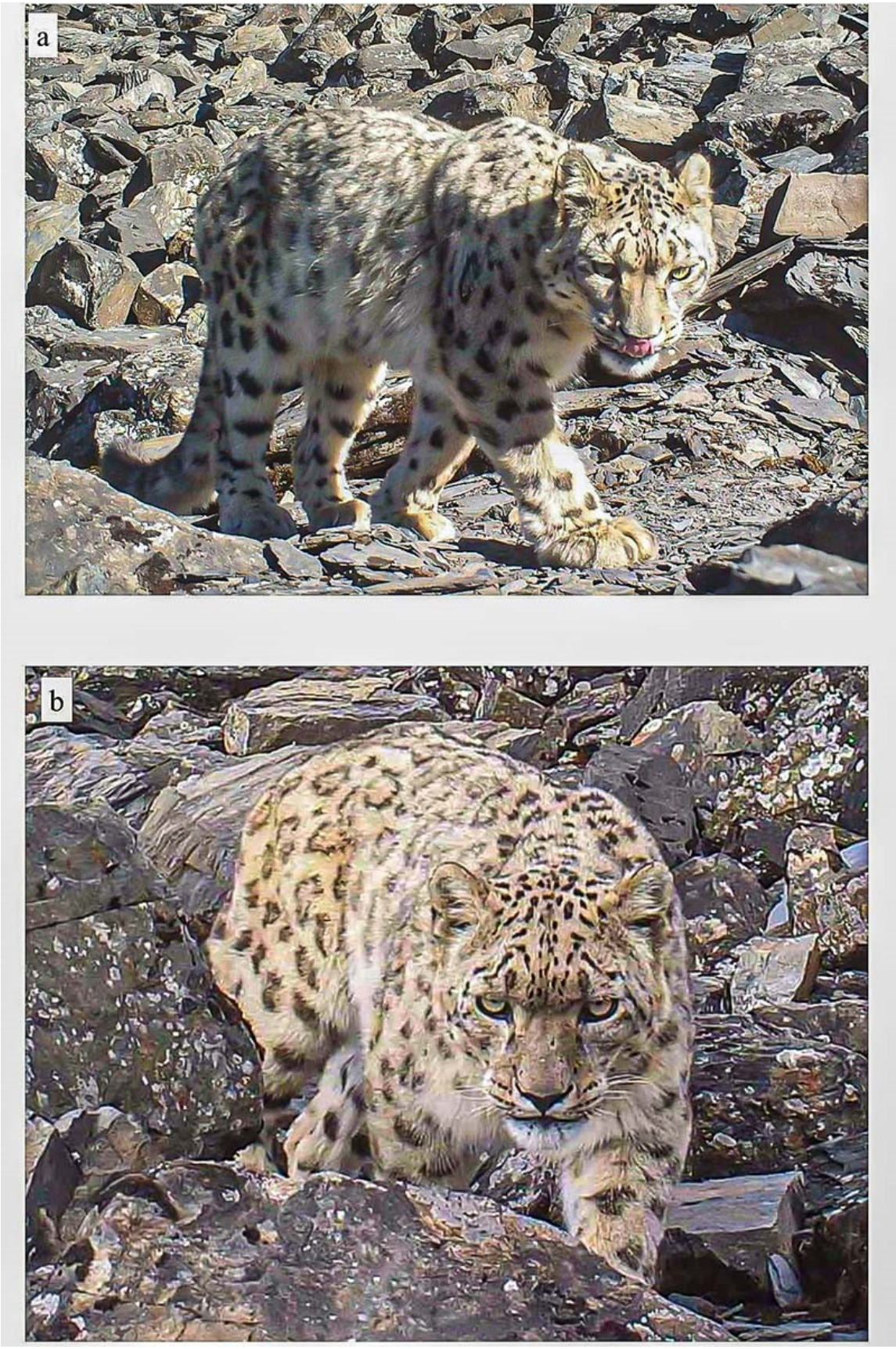


Fig. 5.3 Two different snow leopard individuals captured at the same place by camera trap distinguished by different pelage patterns on forehead and body surface.

We used the program CAPTURE to estimate snow leopard abundance and capture probabilities (Otis et al. 1978; Rexstad and Burnham 1991). The modelling of capture probabilities were performed by assessing the discriminant function model selection for the three likely models: Mo (null model) assumes no variation between individuals or over time, Mh (heterogeneity model) assumes capture probability heterogeneous among individuals due to sex, age, activity, ranging patterns, etc., Mb (behaviour model) assumes capture probability affected by trap response e.g., trap-happy or trap-shy animals and Mbh (heterogeneity and behavior model) is combination of the Mh and Mb models. We used 5-day sampling occasions based on Otis et al. (1978) and following Jackson et al.'s (2006) discussion to fit the recommendations of capture probabilities > 0.10 (and preferably > 0.20) with a sample of > 5 occasions. The program CAPTURE calculates population size, its standard error and 95% confidence interval.

To estimate population density, we divided a total population size obtained from CAPTURE by areas of study areas. Areas of each study areas (LM, UM and SNP) were obtained from total grid cells used for placing camera traps. The areas surveyed were identical with those surveyed in the previous studies. Therefore, our results were unbiased, as far as geographic condition and size of study areas is concerned to compare the status and trend of snow leopard population size over the years.

5.3.3 Prey Survey (Blue Sheep in ACA and Himalayan Tahr in SNP)

We located and counted snow leopard's main local prey (blue sheep in ACA: LM & UM) and Himalayan tahr in SNP) from as many suitable vantage points as possible using 8x21 binocular and 15–45x spotting scopes (Schaller 1977; Jackson and Hunter 1996). In each of the three areas (LM, UM, SNP), we walked all valleys in the area and searched for prey within each valley, divided into several survey polygons based on physical barrier such as river, deep gorge or high mountain, prior to the monitoring. All individuals of blue sheep (in LM and UM) or Himalayan tahr (in SNP) were counted, classified to sex and age class when possible, and their position was marked in the map. Each polygon was scanned during the monitoring walk again and all prey individuals recorded in the same way. We have repeated these monitoring walks several times during each season, in order to avoid the bias caused by the prey herds moving from one site to another one. During each monitoring walk, each valley was scanned within 1 to 3 days to avoid overlapping of previously counted tahr herds and came up with total number of prey. Tahrs, classified to sex and age class, we recorded repeatedly during each monitoring walk and the maximum number of each class of tahr among all monitoring walks were taken to the final count. This way we captured all animal herds in the corresponding valley.

In LM, we monitored blue sheep in four valleys: Thini (Vrapsa and Namu), Lupra, Muktinath and Jhong and in UM in five valleys: Proper Manang, Yak Kharka, Khangsar, Thorang Phedi and Tilicho. These are the same valleys, where the leopard transects were located. Note that valleys were chosen according to the local terrain within each site (LM, UM, SNP) and they are not the same as the Village Development Committees – they even may lay overlap between of these. We categorized blue sheep into young (less than a year), yearling (1 to 2 years), and adult female and male (over 2 years). We further categorized male blue sheep into young male (2 to 4 years), sub adult male (4 to 7 years) and adult male (> 7 years) (Figs. 5.4 to 5.6). We monitored blue sheep in LM (101 km^2) and UM (105 km^2) during the same period, when camera trap surveys were performed.

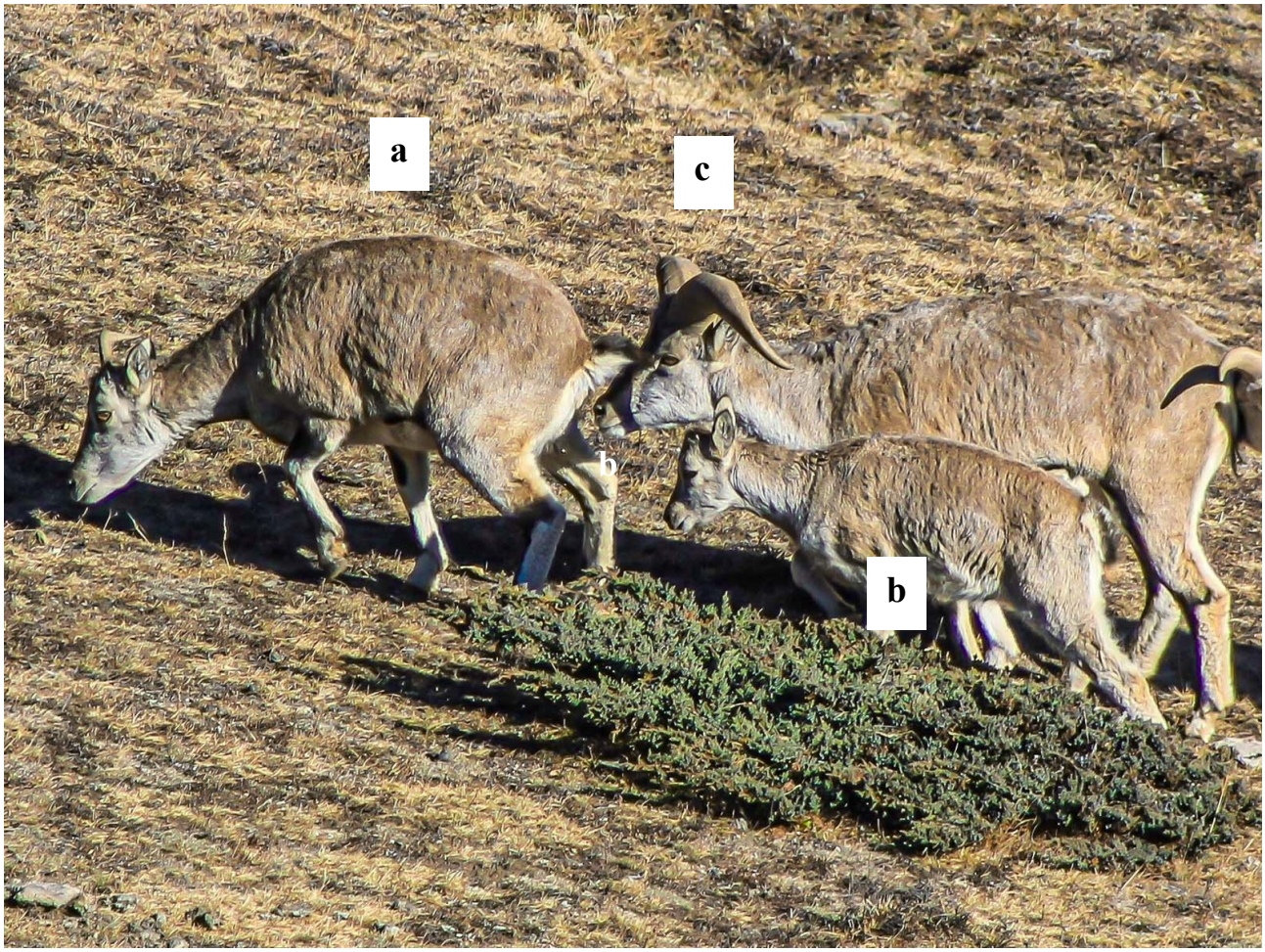


Fig. 5.4 Blue sheep in dry season (November). (a) adult female, (b) young (kid), (c) sub-adult male.

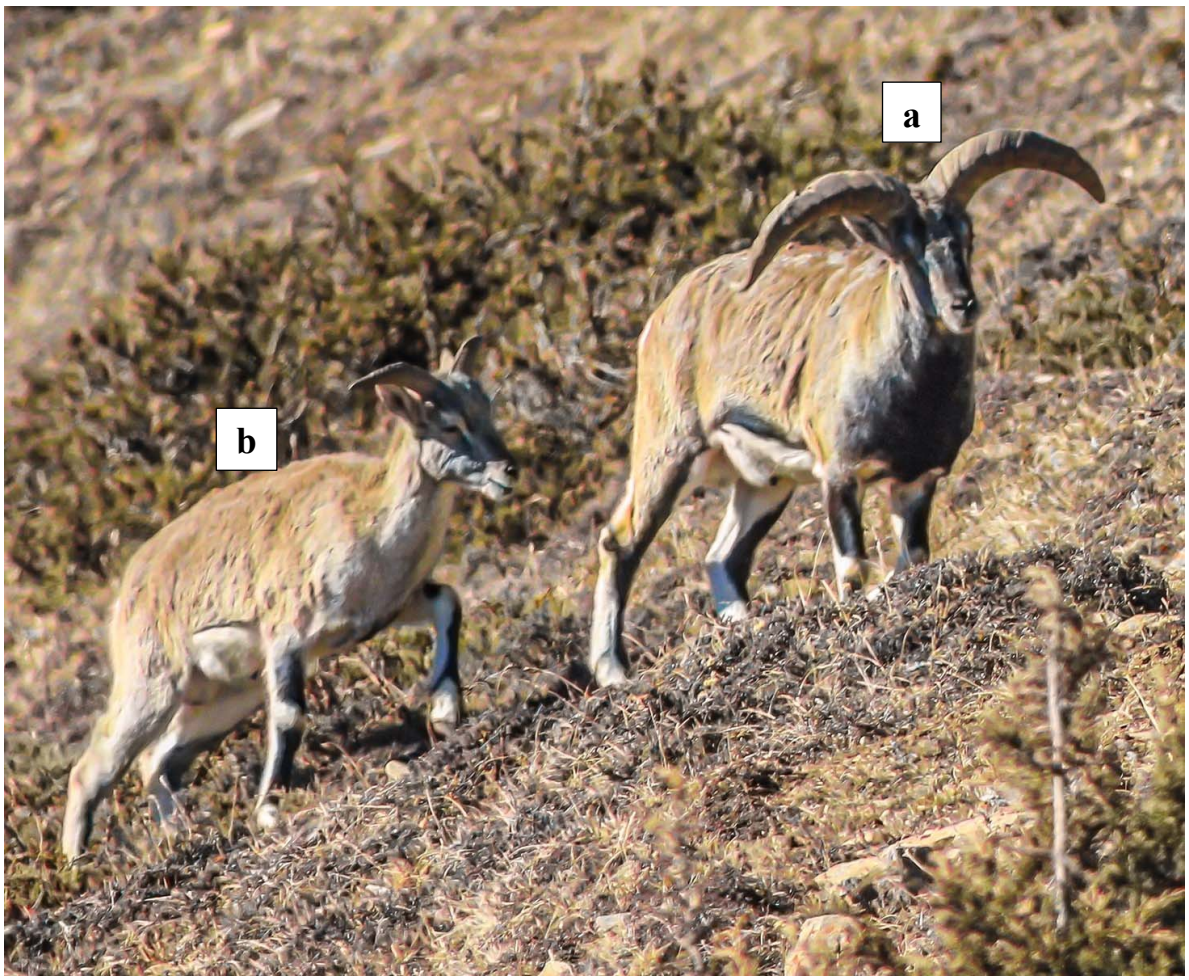


Fig. 5.5 Blue sheep in dry season (November). (a) adult male, (b) young male.

Fig. 5.6 A herd of blue sheep in dry season (November) in Upper Manang of Annapurna Conservation Area.

In SNP, we categorized Himalayan tahr into kids (< 1 year old), yearling (1–2 years), adult female (2 years) and adult males. The adult males were further categorized into class I (2–3 years), class II (3–4 years), class III (5–7 years, dark brown), class IV (7–9 years, pale brown), and class V (9 years, golden or blond), based on mane length/colour, horn length/shape, and body size (Figs. 4.7 to 4.12). The surveys for Himalayan tahr in SNP were carried out in 2004 (June-July), 2006 (Spring: May-June; Autumn: September-October), 2007 (Spring: April-June; Summer: July-September; Autumn: October-December), 2009 (Spring: March-April); Autumn: October-December) and 2015 (same as camera trap survey period). We monitored tahr in four valleys: Gokyo, Phortse, Namche and Thame covering 100 km^2. The total counts reveal population sizes (a minimum number known alive) and population demographic structure.

Mortality rate of blue sheep and Himalayan tahr was calculated as 1- N_t /N_{t-1} where N_t is the number of individuals of this category in time t and N_{t-1} is the number of individuals of the corresponding category in previous time *t-1*.

For Himalayan tahr, mortality rates were calculated for spring period, i.e. before July-August, when kids are born, and for autumn-winter period after the birth of kids. During July and August, when most of kids are born, females often temporarily leave their herd. During these wet months, clouds often reduce visibility and therefore new kids were firstly counted in September. In the same time, last year's year kids were transferred to the category yearlings and last year's year yearlings were transferred to the category female or male category class I. All last year's year kids and last year's year yearlings transfer to the higher demographic category (or die) but males can go to higher demographic category or stay the same category or die. Life cycle of Himalayan tahr is shown in Fig. 5.13.

Fig. 5.7 Adult female Himalayan tahr in dry season (December).

Fig. 5.8 Young (kid) and yearling of Himalayan tahr in dry season (May).

Fig. 5.9 a Male-I Himalayan tahr and **b** Male-II Himalayan tahr in dry season (December).

Fig. 5.10 Dark brown (Class III) of Himalayan tahr in dry season (December).

Fig 5.11 Pale brown (class IV) Himalayan tahr in dry season (December).

Fig 5.12 Golden or blonde (class V) Himalayan tahr in dry season (December).

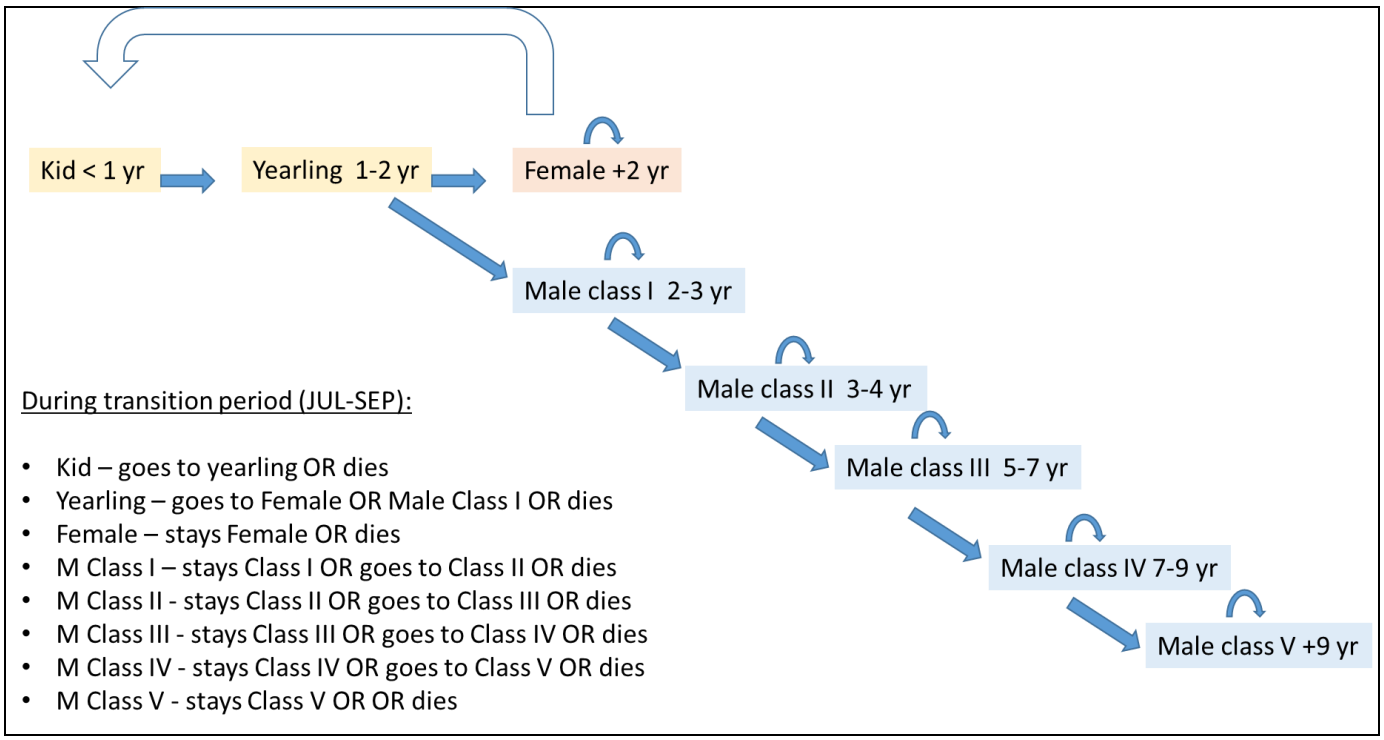


Fig 5.13 Life cycle of Himalayan tahr. Main demographic categories and possible transitions are shown.

5.3.4 Estimation of Parameters of Snow Leopard Population Dynamics

We compared our results of sign monitoring, prey count (blue sheep and Himalayan tahr) and total snow leopard population abundance or density with data obtained by previous researchers or published literature to analyse, how the population fluctuated between years. The areas surveyed were identical with those surveyed in the previous studies. Therefore, our results were unbiased, as far as geographic condition and size of study areas is concerned.

5.4 Results

5.4.1 Non-target animals in the study areas

There are several large or medium carnivore species in the region studied: snow leopard (*Panthera uncia*), Himalayan wolf (*Canis lupus*), Eurasian lynx (*Lynx lynx*) and red fox (*Vulpes vulpes*). Wild ungulate species include blue sheep (*Pseudois nayaur*) and musk deer (*Moschus spp*).

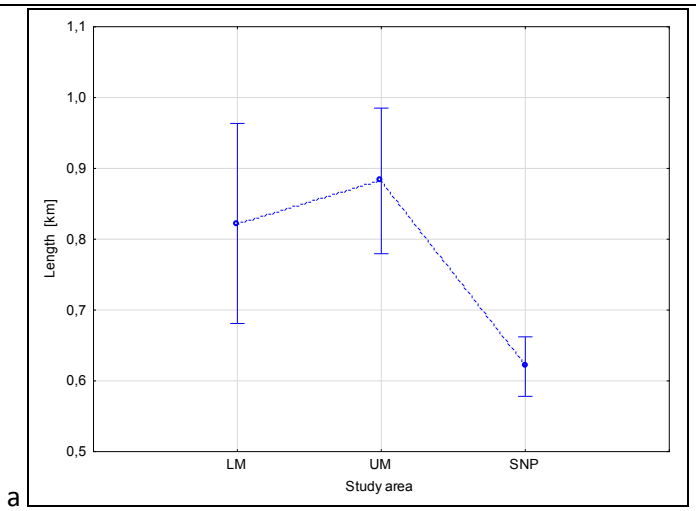
By using camera traps and faecal DNA tests, we detected, besides snow leopard, five other large mammalian predators occurring in this area: common leopard (*Panthera pardus*), Himalayan wolf (*Canis lupus*), Eurasian lynx (*Lynx lynx*), golden jackal (*Canis aureus*) and red fox (*Vulpes vulpes*). Blue sheep (*Pseudois nayaur*) and musk deer (*Moschus chrysogaster*) were the only wild

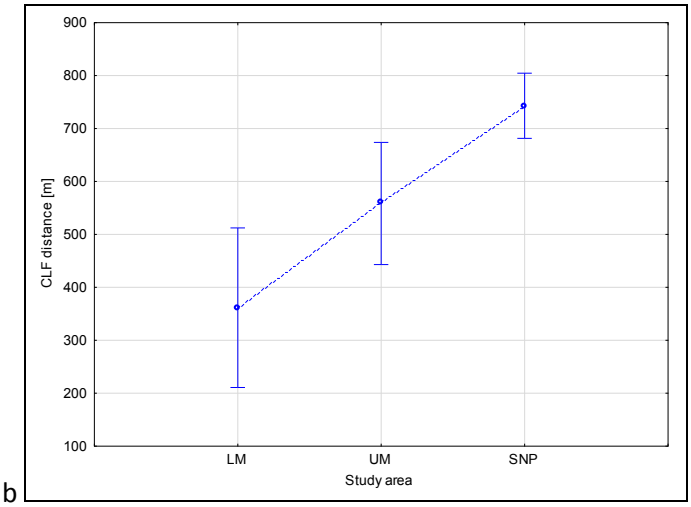
ungulates in this area. Among small mammals, we recorded, Royle's pika (*Ochotona roylei*), least weasel (*Mustela nivalis*), stone marten (*Martes foina*), Sikkim vole (*Alticola sikkimensis*), Pallas's cat (*Otocolobus manul*), jungle cat (*Felis chaus*) and leopard cat (*Prionailurus bengalensis*), all of which are potential prey of snow leopard.

5.4.2 Comparison of the transects parameters

Most of our transects were conducted during dry seasons (SNP in years 2006, 2007, 2009, 2015, LM & UM in 2014), only two transects during wet season 2016 (LM & UM; for more details see Table 5.1). We repeatedly monitored the same transects in SNP but not in LM and UM. This was because we had to establish transects in a lower elevation in LM and UM during dry season 2014 due to high snow layer, and we were not able to use these transects again during wet season 2016, when snow leopards and their prey live in a higher elevation and therefore new transects had to be established. To avoid the possibility that the results of our monitoring were caused by biotope variation (quality) in different years (dry vs. wet season), we tested whether transects studied in different years differed in habitat quality. We also tested differences in transect parameters between SNP and LM and UM of ACA to be able to compare transects established in two study areas, where with different species of snow leopard's prey live.

There were significant differences in lengths of transects, their elevations, distances to cliff and slopes ($P < 0.05$). Transects in SNP were shorter (Fig. 5.14a) and were located in lower elevations than transects in LM and UM (avg. elevation 3898 m a.s.l. in SNP vs. 4186 m a.s.l. in LM & UM). SNP transects distances to nearest cliff were significantly larger (Fig. 5.14b, Table 5.1) and were not as steep as transects in UM and LM (Fig. 5.14c, Table 5.1). Differences between transects monitored in SNP and LM and UM of ACA reflected differences in geomorphology of two study areas. We found no significant differences in these parameters among transects conducted in SNP, LM and UM when we analysed only 9 LM transects and 17 UM transects monitored during dry seasons 2014 and SNP transects, which all were studied during dry seasons.





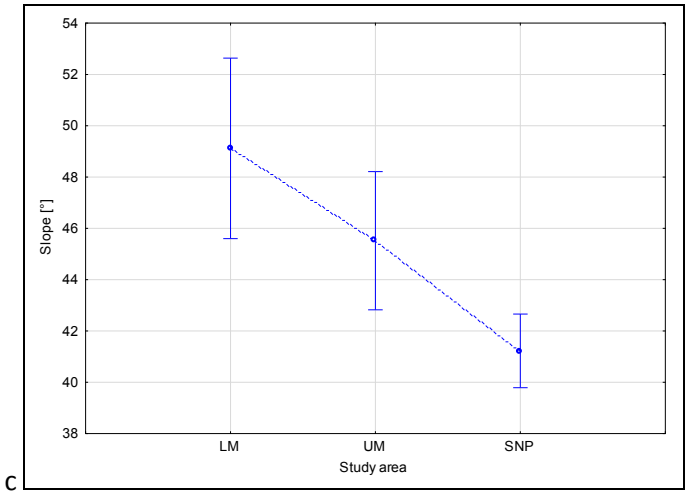


Fig. 5.14 Topographic parameters of transects investigated in three study areas (LM - Lower Mustang, UM - Upper Manang, SNP - Sagarmatha National Park) during dry seasons. (a) The length of transects: transects in SNP were significantly shorter than transects in LM & UM. (b) Distance of transects from the nearest cliff: transects in LM had the shortest distances to the cliff, whilst transects in SNP were located in largest distances from cliffs. (c) Slope of transects: transects in LM were the most steep, whilst transects in SNP were the least steep. Vertical bars denote 0.95 confidence intervals.

Fig. 5.15 Proportions of topography types (a), habitats (b) and ruggedness types of transects (c) investigated in three study areas (LM – Lower Mustang, UM – Upper Manang, SNP – Sagarmatha National Park) during dry seasons. (a) Four topography types were distinguished: TopoRID – ridges, TopoHS – hill slopes, TopoBOL

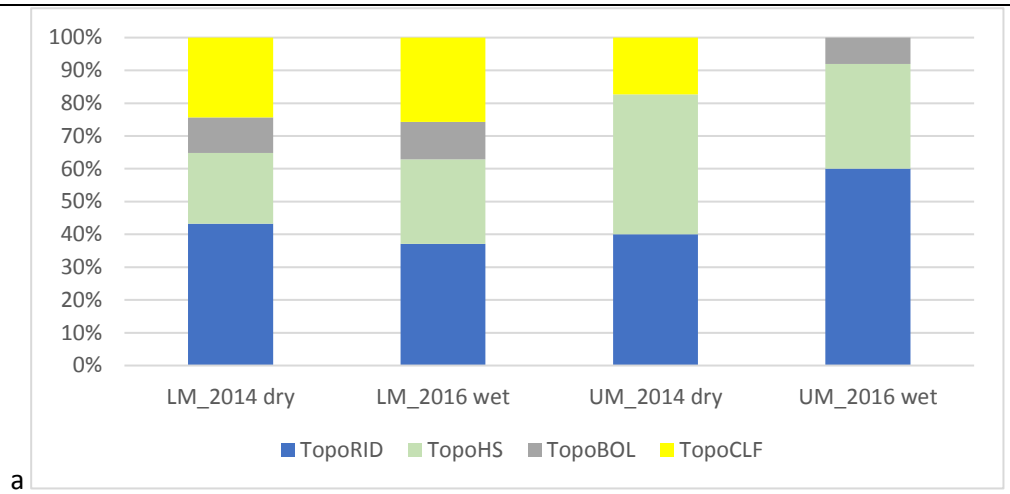
– boulders, TopoCLF – cliffs. (b) Six habitat types were distinguished: VegG – grassland, VegOF – open forest, VegS – shrubland, Veg SC – scrubland, VegF – forest, VegB - barren. (c) Three types of ruggedness were distinguished: RugBK – terrain broken, RugROL – terrain rolling, RugVB – terrain very broken. For detail description of distinguished parameters – see Table 5.1.

When we compare dry season transects from SNP and dry season transects from LM & UM, we found differences in proportions of different topography (Fig. 5.15a), habitat (Fig. 5.15b), and ruggedness types (Fig. 5.15c). Hill slopes (topography type HS) was the most common topography type in all dry season transects at all. HS covered about 75% of transects in SNP, but only 21.6% of dry season transects in LM and 42.7% of dry season transects in UM. Ridges (topography type RID) were more common in LM & UM than in SNP and cliffs (topography type CLF), frequent in LM & UM and almost absent in SNP transects. Shrublands (vegetation type S) were the most common vegetation type occurring in dry season transects. This vegetation type covered more than 55% of transects in SNP, 50% in UM but only 35% of transects in LM. Open forests (vegetation type OP), the second most common vegetation type in SNP, covered only 10% of dry season transects in LM and was missing in UM. Scrublands were recorded only in LM transects (Fig. 5.15b). Rolling terrain (ruggedness type ROL) was recorded on more than 50% of transects in SNP. The same ruggedness type covered nearly 70% of dry season transects in UM but only 35% in LM. Very broken terrain (ruggedness type VB) was missing or very rare in SNP transects but covered more than 20% of transects in LM and over 10% transects in UM (Fig. 5.15c).

We planned to compare behaviour of snow leopard and its prey in summer and winter, so we wanted to make sure that the differences were not affected by transect parameters and we tested for differences between transects monitored in LM and UM during dry season 2014 and wet season 2016 (Fig. 5.16). We found no significant differences in topography types between dry and wet season transects in LM but there were differences in dry and wet season transects in UM. Cliffs (topography type CLF) covering more than 17% of dry season transects in UM were missing in transects monitored in UM during wet season 2016 (Fig. 5.16a). Whilst topography types recorded during dry and wet seasons in LM were very similar to each other, transects significantly differed in vegetation types (Fig 5.16b). Shrubland (vegetation type S) covering more 35% of dry season transects in LM was completely missing in transects monitored during wet season in LM. Also proportions of other vegetation types were different (Fig. 5.16b). Similarly, broken terrain (ruggedness type BK) covering more than 40% of dry season transects in LM was missing in wet season transects monitored in LM. Proportion of three ruggedness types slightly differed between transects conducted during wet and dry season in UM (Fig. 5.16c).

In SNP, transects were located in four valleys, that significantly differ in main topographic parameters (One-way ANOVA, $P < 0.05$; Fig. 5.17). They also differed in habitat types and types of

ruggedness. Transects located in different valleys in UM and LM not differed significantly in their topographic parameters. Only transects in Lupra valley (LM, wet season 2016) were slightly longer and transects in Jhong valley (LM, both dry season 2014 and wet season 2016) were steeper and closer to cliffs. But none of these differences were statistically significant (One-way ANOVA, $p > 0.05$).



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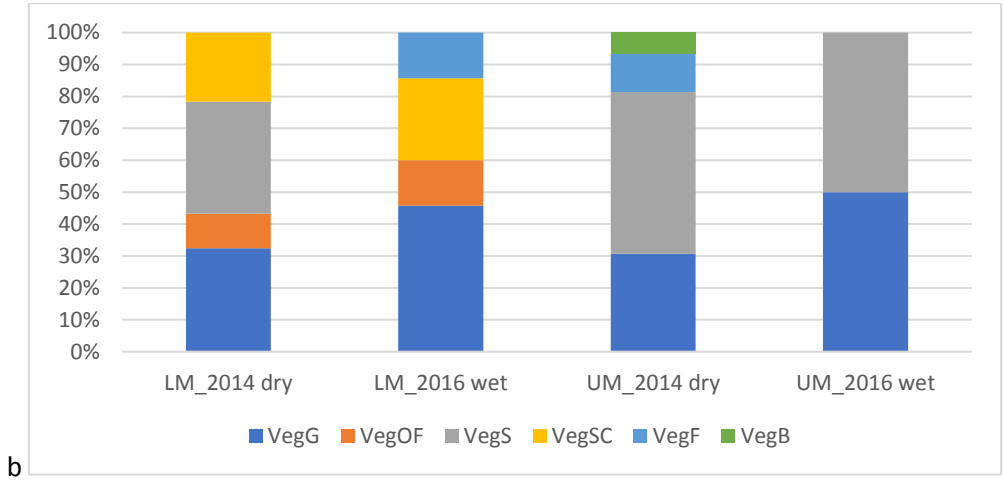

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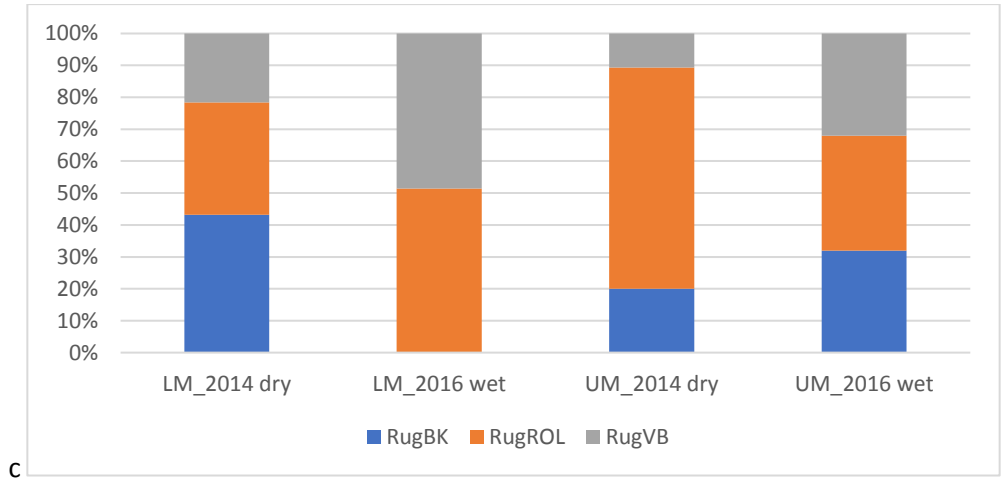

c

Fig. 5.16 Proportions of topography types (a), habitats (b) and ruggedness types (c) of transects investigated in Lower Mustang (LM) and Upper Manang (UM) during dry and wet seasons. (a) Four topography types were distinguished: TopoRID – ridges, TopoHS – hill slopes, TopoBOL – boulders, TopoCLF – cliffs. (b) Six habitat types were distinguished: VegG – grassland, VegOF – open forest, VegS – shrublands, Veg SC – scrubland, VegF – forest, VegB - barren. (c) Three types of ruggedness were distinguished: RugBK – terrain broken, RugROL – terrain rolling, RugVB – terrain very broken. For detailed description of distinguished parameters – see Table 5.1.

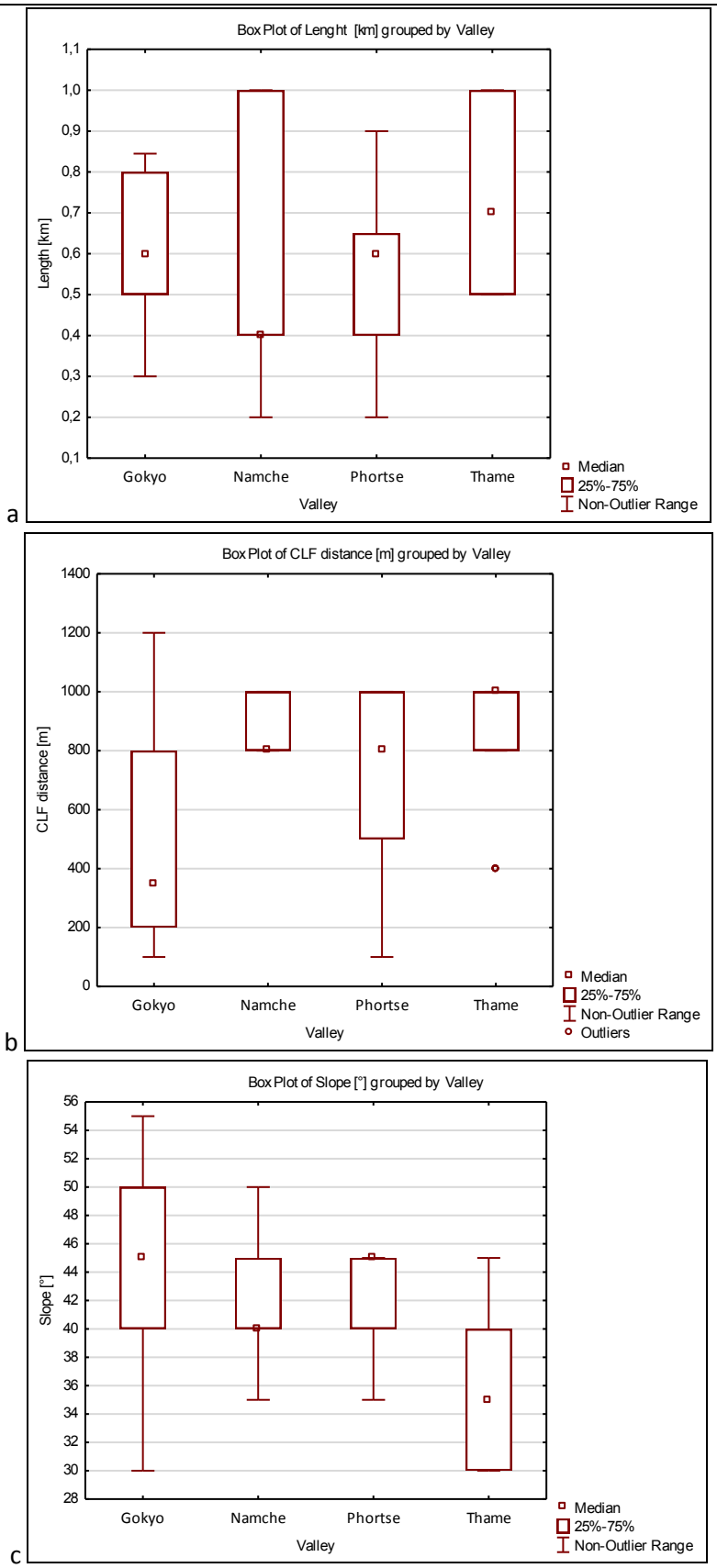

Fig. 5.17 Differences of main topographic parameters of transects located in four valleys of Sagarmatha National Park. (a) The length of transects, (b) Distance of transects from the nearest cliff, (c) Slope of transects.

We can briefly summarize that our study transects significantly differed in many of their parameters when data about all transects and data about transects monitored during dry seasons were included. We found no significant differences in length of transects, elevation and slope among transects conducted in SNP, LM and UM when we analysed only transects monitored during dry seasons 2014 (LM & UM) and 2015 (SNP). Also other parameters differed only slightly and statistically significant differences were not found for data from dry seasons 2014 and 2015.

5.4.3 Indicators of Snow Leopard Abundance and Population Size

We considered two main indicators of snow leopard abundance: sign encounter rates (number of scrapes/km in this paper) and relative abundance indices (RAI – see Methods for formulas of their calculation).

The ***sign encounter rates*** within the ACA were higher in UM than in LM. For example, in summer 2016 it was 5 scrapes/km in UM whilst only 2.5 scrapes/km in LM (Fig. 5.18). However, this difference was not statistically significant (Table 5.3). Number of scrapes recorded during dry period 2014 were significantly higher than number of scrapes recorded during wet period.

Table 5.3 Results of ANOVA of number of scrapes/km recorded in two study areas (LM – Lower Mustang, UM – Upper Manang) during dry and wet period (2014 and 2016 resp.). Significant p-values are marked by*.

Effect	SS	DF	MS	F	p
Study area	24.43	1	24.43	2.384	0.130
Period	43.05	1	43.05	4.202	0.047*
Study area*Period	15.07	1	15.07	1.470	0.232

The sign encounter rates were higher in ACA than in SNP, when all data were considered from 2014–2016 (t-test, $t = 5.512$, $P < 10^{-7}$; Fig. 5.18). In SNP, the snow leopard sign encounter rates were gradually decreasing from 2006 to 2009 ($R = 0.385$, $P < 0.0003$) and then increased in 2015, when data from 2009 and 2015 were compared. (t-test, $t = -2.32$ $P < 0.05$).

In all valleys of the study areas, snow leopard signs were recorded during the study periods except in Thame valley of SNP and Lupra valley of LM, where the last signs were recorded in 2010. Among the four valleys of SNP, no snow leopard signs were found in Thame in both seasons of 2015 whilst the sign encounter rate monotonously decreased from 2005 to 2009 in this area. There were

significant differences between the four valleys, years and valley*year interaction in number of recorded scrapes (Table 5.4, Fig. 5.19).

Table 5.4 Results of ANOVA of number of scrapes/km recorded in four valleys of SNP in years 2006, 2007, 2009 and two periods of 2015. Significant p-values are marked by *.

Effect	SS	DF	MS	F	p
Year	55.49	4	13.87	4.037	0.004*
Valley	45.34	3	15.11	4.398	0.006*
Year*Valley	91.18	12	7.60	2.211	0.016*

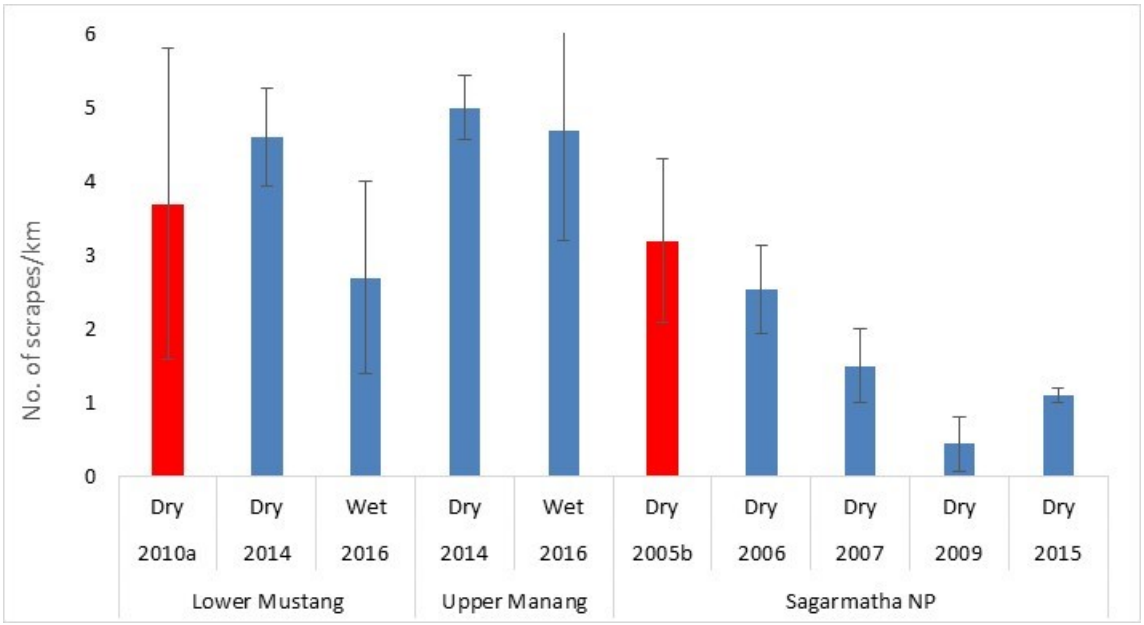

Fig. 5.18 Sign encounter rates (numbers of scrapes/km) in three regions from 2005 to 2016. Indicators next to the year (red columns) indicate the data were not collected in this study – the sources were: a – Ale and Shrestha et al. 2014, b – Ale 2007, years without sign – data collected in this study. Dry = dry season and wet = wet season. The vertical bar lines above the bar diagrams indicate standard errors.

Surprisingly, snow leopard signs were found in some of Gokyo transects in season 2015, while there were no sings in 2006 and these transects were not investigated in 2007 and 2009 (Table 5.1). Less snow leopards' signs were recorded in Namche valley in 2015 than in 2006. There was a significant decrease between 2006 and 2009 and a slightly increase from 2009 to 2015, when more signs were recorded during spring dry season than during autumn dry season (Fig. 5.19). On the contrary, more leopards' signs were recorded during dry autumn season 2015 than during spring dry season 2015 in Phortse valley.

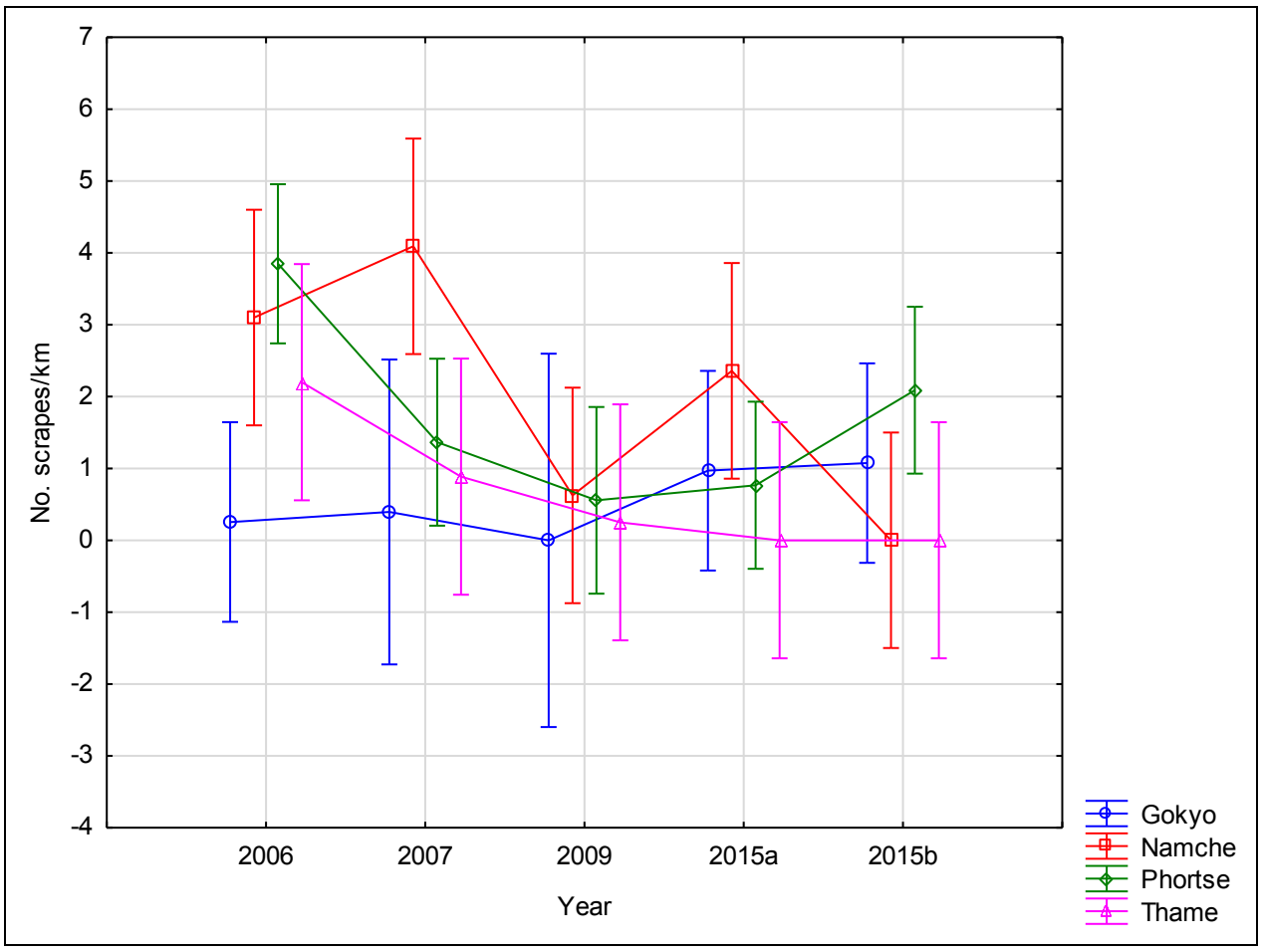

Fig. 5.19 Number of scrapes/km recorded in four valleys of Sagarmatha National Park during the five monitoring periods. Interaction year*valley are shown. Letters a/b next year 2015 indicate (a) – spring dry season 2015 and (b) – autumn dry season 2015. Vertical bars denote 0.95 confidence intervals.

Snow leopard ***relative abundance indices*** (RAI) were obtained from camera trap data (Table 5.5). The largest RAI was found in LM in winter of 2014 and in UM in autumn of 2014 (4.3 and 4.7 respectively). However, RAI was lower in LM than in UM in summer of 2016. Thus, RAI was not consistently lower in UM. Note also the drastic decrease of RAI in LM from winter 2014 to summer 2016. RAI was consistently lowest in SNP, compared with LM and UM. Snow leopards were not captured in Thame valley in either of the seasons of 2015 in SNP and in Lupra of LM of both years 2014 and 2016. This corresponds to the lack of any sign of presence of snow leopard (scrapes) during this period in Thame and Lupra valley.

There were many ***non-target images*** in the data. They included livestock, human, bird species, common leopard, Himalayan wolf, red fox, golden jackal, Eurasian lynx, jungle cat, leopard cat, pika, stone marten and Himalayan weasel. Common leopards were recorded (capture events = 3) in alpine grassland at 4300 m in LM and in shrubland above tree line in SNP (capture events = 8). In lots of captures, no animals were visible. This was because the camera was triggered by moving grass and cloud, by changing sun orientation and its sunrays or so. Such images are called ***false images*** here.

As a result, there is a notably low percentage of snow leopard captures among all captures made (0.2 to 2.6%).

5.4.3 Numbers of Snow Leopard Individuals Captured by Camera Traps

In LM, six adult snow leopard individuals were captured by cameras in 2014: two adult males, two adult females (one of the females had one cub), one sub-adult snow leopard and two adults were of unknown sex. Three individuals which were observed in 2010 (Ale et al. 2014) were also recaptured in 2014. In 2016, only one adult male was recaptured from 2014 records along with two new adults, out of which one was male and the other was unidentified but those three individuals captured in 2010 and 2014 were not captured in 2016. Thus, eight individuals were observed during the 2014 and 2016 monitoring.

In 2014, eight adult individuals plus two cubs were captured in UM: one adult male, one adult female with two cubs, and six adults were individuals of unidentified sex. In 2016, only six individuals were recaptured (one adult female and one adult male, and the other individuals were of unknown sex) and no new individuals were found. Thus, eight individuals were observed in UM during the monitoring in 2014 and 2016. In SNP, we identified three adults of unknown sex in spring 2015, out of which two individuals were recaptured; no new individuals were observed in autumn 2015. Thus, three individuals were observed in SNP during 2015.

Based on the capture and recapture method (Otis et al. 1978), there is consistent abundance of snow leopard among three models in LM (Mo, Mh, Mb) with capture probability 0.5–0.67. In case of UM, Mo and Mh models are weakly fitting the data (capture probability 0.1–0.13 and large variation of the 95% confidence interval), while Mb model fits the data closely with capture probability 0.5 and small variation of 95% confidence interval. In case of SNP, two models (Mo and Mb) are the best fit with capture probability 0.3, while Mh model fits the data weakly (capture probability 0.09). In Mbh model, capture probability was not computed in all three areas (Table 5.6). Based on the best-fit selection model, the calculated population sizes in LM, UM and SNP were 6, 8 and 3 respectively.

Table 5.5 Results of camera traps in three study areas in 2014, 2015 and 2016.

	Lower Mustang		Upper Manang		SNP	
Detail	2014 Dry season	2016 Wet season	2014 Dry season	2016 Wet season	2015 Dry-spring	2015 Dry-autumn
Trapping effort (trap nights)	414	1020	321	1000	557	1215
Full images (SL)	132	34	43	53	25	27
Partial images (SL)	122	29	43	53	26	24
Total images (SL)	254	63	86	102	51	51
Capture events	22	12	15	24	12	14
False images	7130	2610	13154	2632	13510	19022
Non target images (non-target)	2439	4100	1619	2373	2462	10816
Percentage of SL captures	2.6	0.9	0.6	2.0	0.3	0.2
No. of captures/100 nights (RAI)	5.3	1.2	4.7	2.4	2.2	1.15
Number of individuals	6	3	8	6	3	2

Table 5.6 Estimated abundances and capture probabilities of snow leopards sampled following the method Otis et al. 1978 in Lower Mustang (LM), Upper Manang (UM) and Sagarmatha National Park (SNP) in 2014–2015. The bold numbers are the best fit among the three models (Mo, Mh, Mb), (± SE) is the standard error. The range values in parentheses just below the abundance are 95% confidence intervals.

	Test for closure	**Based on Mo (Null)**		**Based on Mh (Heterogeneity)**		**Based on Mb (Trap Response)**		**Mbh (Heterogeneity and Trap Response)**	
Study area	CAPTURE	Capture probability	Abundance ± SE 95% C. l.	Capture probability	Abundance ± SE 95% C. l	Capture probability	Abundance ± SE 95% C. l	Capture probability	Abundance ± SE 95% C. l
LM	z = -0.026 P = 0.489	0.5	6 ± 0.32 (6–6)	0.50	6 ± 0.50 (6–6)	**0.67**	**6 ± 0.09** **(6–6)**	Not computed	6± 0.09 (6–6)
UM	z = -0.894 P = 0.1855	0.1	25 ± 19.45 (11–110)	0.13	17 ± 5.0 (12–33)	**0.50**	**8 ± 1.1** **(8–8)**	Not computed	8± 1.1 (8–8)
SNP	z = -0.488 P = 0.312	**0.3**	**3 ± 0.89** **(3–3)**	0.09	9 ± 3.7 (5–21)	0.29	3 ± 1.2 (3–3)	Not computed	Not computed

5.4.4 Population Density per 100 km^2 and its Estimation by Sign Encounter Rate and RAI

Snow leopard density has slightly increased in UM from 1991 to 2014–2016, decreased in LM from 2014 to 2016, and decreased in SNP from 2006 to 2010–2015 (see Table 5.7).

Table 5.7 Population size and density of snow leopard in LM and UM and SNP in different years.

Location	Year	Population size	Density of snow leopard per 100 km^2
UM	1991[a]	5	4.8–6.7
	2014	8	7.6
	2016	6	5.7
LM	2014	6	6
	2016	3	3
SNP	2005[b]	6	6
	2010[c]	2	2
	2015	3	3

Indicators next to the year: a – Oli 1994, b – Lovari et al. 2009, c – Ferreti et al. 2014, without sign – this study.

There was a significant positive correlation ($R^2 = 0.85$) between sign encounter rates (No. of scrapes/km) and estimated density of snow leopard per 100 km^2 (Fig. 5.20). Likewise, there was a significant positive correlation ($R^2 = 0.66$) between relative index of abundance (RAI) and density of snow leopard per 100 km^2 (Fig. 5.21). The close correlations indicate that both the No. of scrapes/km and the No. of captures/100 nights are good approximations of the actual density of snow leopard.

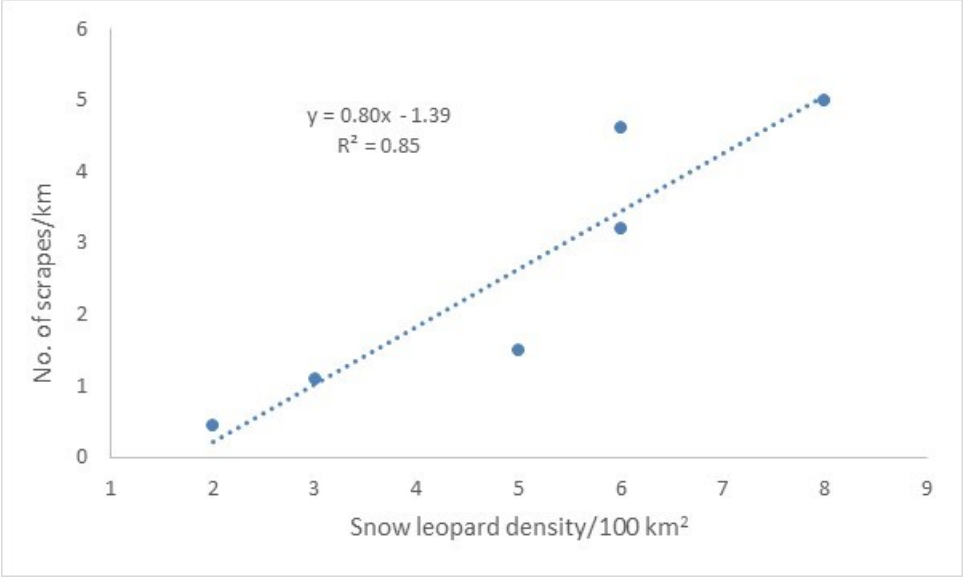

Fig. 5.20 Correlation between sign encounter rate (No. of scrapes/km) and snow leopard density per 100 km^2 obtained in LM, UM and SNP from 2014 to 2015.

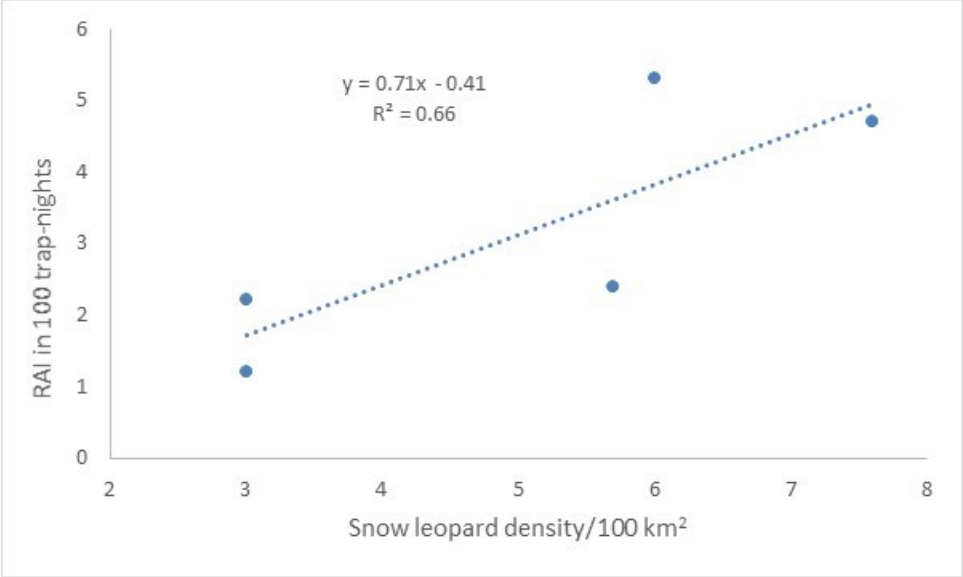

Fig. 5.21 Correlation between relative index of abundance (RAI = No. of captures/100 nights) and snow leopard density per 100 km^2 obtained in LM, UM and SNP from 2014 to 2015.

5.4.5 Population Dynamics of Blue Sheep in Lower Mustang and Upper Manang

Numbers of individuals of blue sheep in LM are shown in Fig. 5.22 and those in UM in Fig. 5.23, together with their density per km^2. Numbers of individuals of blue sheep occurring in four valleys of LM during of dry season 2014 and wet season 2016 differed. Numbers from wet season 2016 were 30% lower (Table. 5.8). There were also significant differences in numbers of blue sheep found in three valleys of UM during of dry season 2014 and wet season

2016, but differences of total number of blue sheep in UM were not significant. Numbers from wet season 2016 were only 10% lower than numbers from dry season 2014 (Table 5.8). The biggest decrease was recorded in Proper Manang Valley, where 128 blue sheep were in dry season 2014 and only 49 individuals in wet season 2016. On the contrary, numbers of blue sheep recorded in Yak Kharka valley increased from 288 in dry season 2014 to 411 in wet season 2016.

Table 5.8 Numbers of blue sheep recorded in four valleys of Lower Mustang (LM) and three valleys of Upper Manang (UM) during dry season 2014 and wet season 2016.

LM	valley	Jhong	Lupra	Muktinath	Thini	**Total**
	Dry season 2014	16	32	16	127	191
	Wet season 2016	45	5	65	17	132
UM	valley	Proper Manang	Yak Kharka	Khangsar		Total
	Dry season 2014	128	288	328		744
	Wet season 2016	49	411	242		702

Linear regression of the dependence of the population density of blue sheep on time (not depicted) yielded the following equations: $y = -0.23x + 2.2$ with $R^2 = 0.21$ for LM and $y = 6.83$ with $R^2 = 0.00$ for UM; neither of the values of R^2 is significant ($P < 0.001$). Thus, the lack of significant correlation between the population density and time indicates that there was no significant trend in population density of blue sheep either in LM or in UM (see also Figs. 5.22 and 5.23).

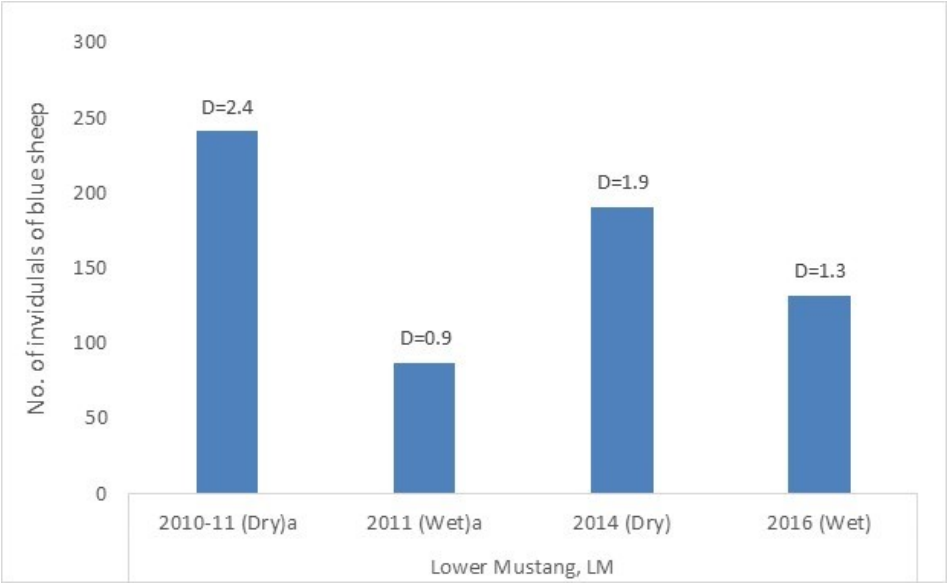

Fig. 5.22 Total population of blue sheep in Lower Mustang. Season indicated as "Dry" or "Wet". D (above the columns) = density, No. of individuals/km^2. Letters next to the year: a – Ale and Shrestha et al. 2014, no letter – data from this study.

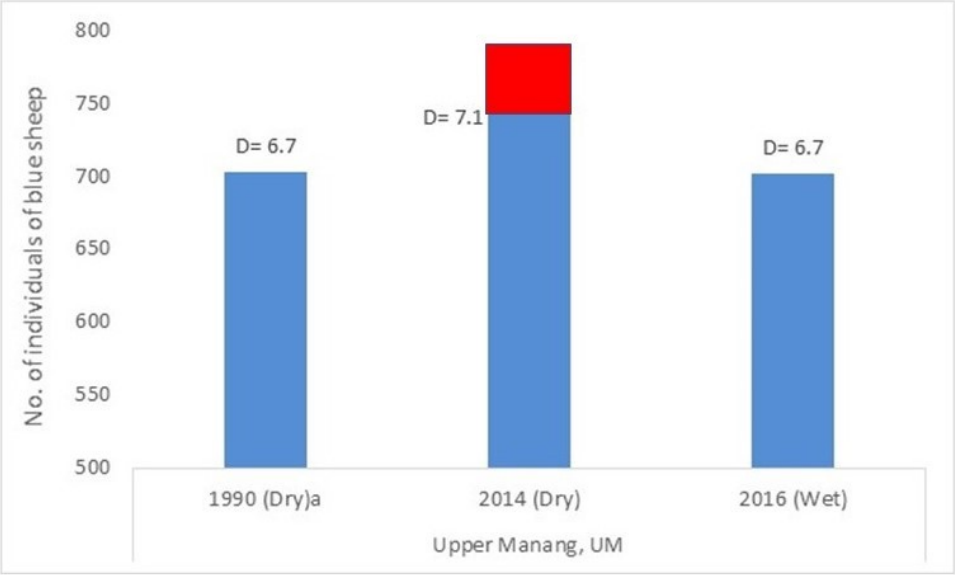

Fig. 5.23 Total population of blue sheep in Upper Manang. Season indicated as "Dry" or "Wet". D (above the columns) = density, number of individuals per km^2. Letters next to the year: a – Oli 1991, no letter – data from this study, The red bar on top of the column 2014 illustrates 50 killed blue sheep individuals by heavy snowing which occurred during monitoring period (see the main text for explanation).

In LM, the sex ratios of male and female were 1.1:1 and 0.62:1 in 2014 and 2016 respectively whilst in UM the ratios were 0.94:1 and 1.1:1 in 2014 and 2016 respectively. In 1990–1991, the mortality rate of blue sheep from autumn to winter in UM was the highest for yearling (0.46), followed by young male mortality (0.41), adult male (0.40), sub adult male (0.35) and female (0.10) mortalities (Oli 1991). In LM, the kid to female ratio (reproductive rate) was 0.54 during dry seasons 2010 and 2011 (n = 29, SE =0.11), 0.55 during wet season 2011 (n = 5, SE = 0.16), 0.5 during dry season 2014 (n = 28, SE = 0.07) and 0.6 during wet season 2016 (n = 7, SE = 0.06), which indicates a stable population growth. There is a lack of correlation between population size of blue sheep and time in LM (Fig. 5.22).

In UM, the kid to female ratio was 0.7 during dry season 1990 (n = 38) (Oli 1991), 0.66 during dry season 2014 (n = 70, SE = 0.042) and 0.65 during wet season 2016 (n = 25, SE = 0.03), which indicates that the population increased slightly over the years. However, the population density is more or less constant over the years in UM (Fig. 5.23). This discrepancy is maybe because of the rather high mortality, for example during the monitoring period in 2014, heavy snowing and avalanches killed ~50 adult male blue sheep in UM (Fig. 5.23).

5.4.6 Population Dynamics of Himalayan tahr in the Sagarmatha National Park

A total population size of the Himalayan tahr recorded during dry-autumn periods decreased from about 350 individuals in 1989 to about 134 individuals in 2009 and after that the population increased to about 223 individuals in 2015 (Fig. 5.24). Similar trends show also population densities (Fig. 5.25). The sex ratios of male to female were 0.88, 0.71, 0.53 and 0.70 in spring of 2006, 2007, 2009 and 2015 respectively.

The reproductive rate (ratio of kid to female) of the tahr population was satisfactory in 1991 (Lovari et al. 2009). Once the snow leopards have been recorded since 2004, the reproductive rate continuously decreased until 2007, and it increased slightly in 2009 but still was lower than in 1991. In 2004 to 2015, the ratio of kid to female ranged from 0.22 to 0.34 during wet season (birthing period), 0.22 to 0.37 in dry-autumn (rutting period) and 0.10 to 0.28 in dry-spring period (before birthing; Fig. 5.26). In 2007, the kid was recorded 43 in September just completing birthing period, which was decreased to 11 (decreased by 74%) in November. Overall, the low reproductive rate (below 0.37) indicates that the population of Himalayan tahr tends to decrease or may eventually vanish over the years in SNP.

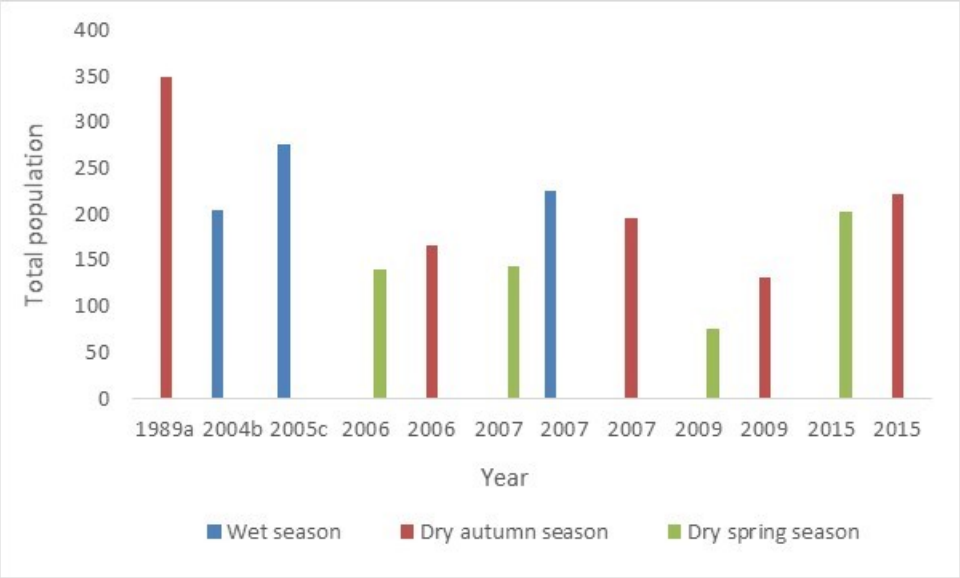

Fig. 5.24 Total population of Himalayan tahr in different years and seasons. Letters next to the year mean the source of data: a – Lovari 1992 and Lovari et al. 2009, b – Shrestha 2006, c – Ale 2007, years not followed by any letter are data from this study. During our study, one, two or three monitoring sessions of tahr population were conducted during each season (i.e. wet season, dry autumn season, dry spring season). In case of multiple sessions, the final number was calculated from the highest number recorded in each tahr demographic category (see Table 5.10 and Fig. 5.27 for multiple monitoring sessions in each season).

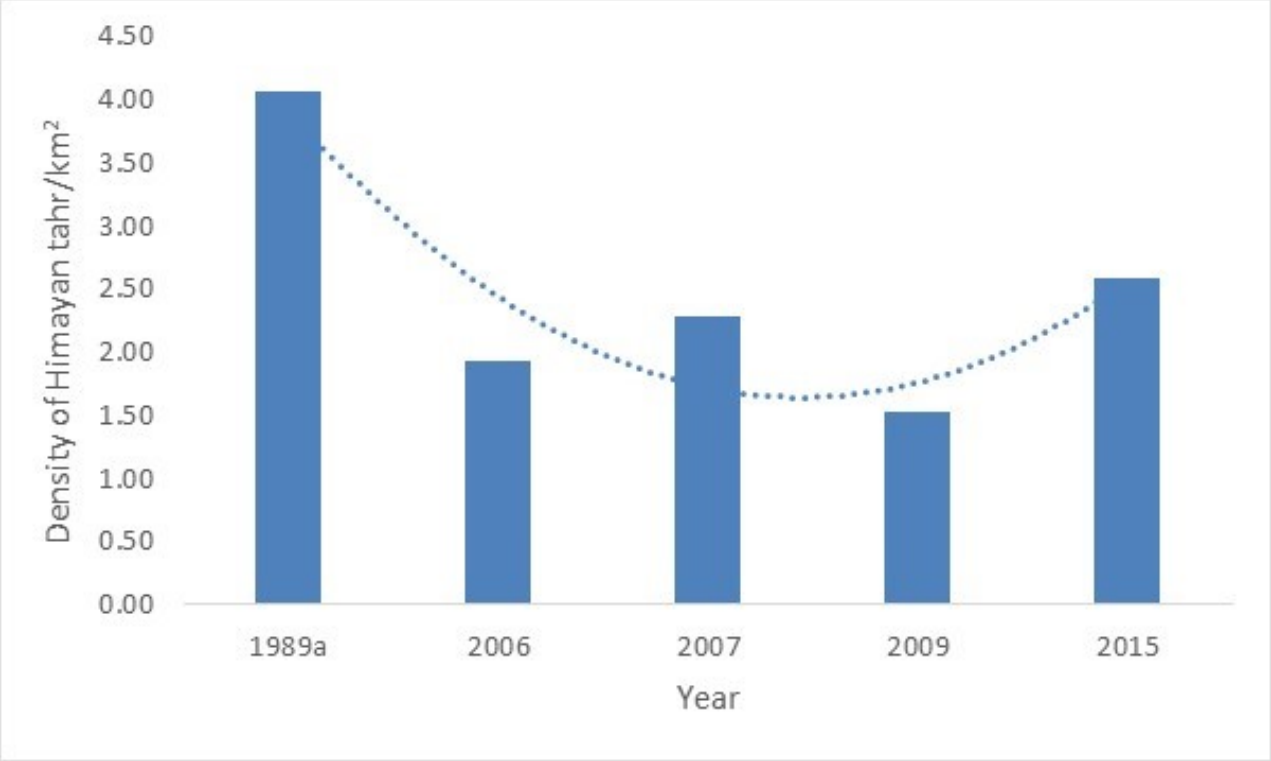

Fig. 5.25 Himalayan tahr density per km^2 in dry season of different years from 1989 to 2015 in SNP. Letter a next to the year means data from Lovari 1992, no letter means data from this study.

Himalayan tahr populations observed in four valleys of SNP in years 2006–2015 significantly differed in their sizes (One-way ANOVA, P <0.001, Table 5.9). The largest tahr population was recorded in Phortse valley, where almost 100 individuals were observed during study period. The smallest population with only about 20 tahrs lived in Thame valley.

Table 5.9 Numbers of Himalayan tahr recorded in four valleys of SNP in years 2006–2015.

Valley	Gokyo	Namche	Phortse	Thame	Total
Dry season 2006	25	53	84	20	182
Dry season 2007	30	64	101	21	216
Dry season 2009	13	40	66	15	134
Dry spring season 2015	15	70	96	22	203
Dry autumn season 2015	16	86	97	24	223

In all four valleys the lowest numbers of tahrs were recorded in 2009 but different trends of tahr populations dynamics were recorded in individual valleys. A small population of tahrs living in Thame valley was more less stable. Numbers decreased from 20 animals in 2006 to 15 ones in 2009 and increased again to 22 respectively 24 in 2015 (Table 5.9). A similar trend but with bigger fluctuations among years were found in tahr population living in

Namche. Numbers decreased from 53 in 2006 to only 40 tahrs in 2009 and almost doubled to 70 respectively 86 individuals recorded in 2015. In Thame and Namche valleys, tahr numbers increased during our study period while signs of snow leopards decreased or even vanished in 2015 (Table 5.9 and Fig. 5.19). In Gokyo valley, numbers decreased from 30 individuals recorded during dry season 2007 to only 30 and 13 tahrs recorded during dry season 2007 and 2009 respectively. This population grew up only slightly and 15 respectively 16 tahrs were observed in 2015. We can assume that the tahr numbers were decreasing in Gokyo while signs of snow leopard were increasing. In case of Phortse valley, the tahr numbers have decreased from 2007 to 2009 and again increased from 2009 to 2015 while the signs of snow leopard decreased from 2006 to 2009 and start to increase from 2009 to 2015 (Table 5.9 and Fig. 5.19). Tahr population density rapidly decreased in 2009, probably because a higher number of predators during the previous years, as indicated by predator presence signs observed (Fig.5.19, Table 5.7).

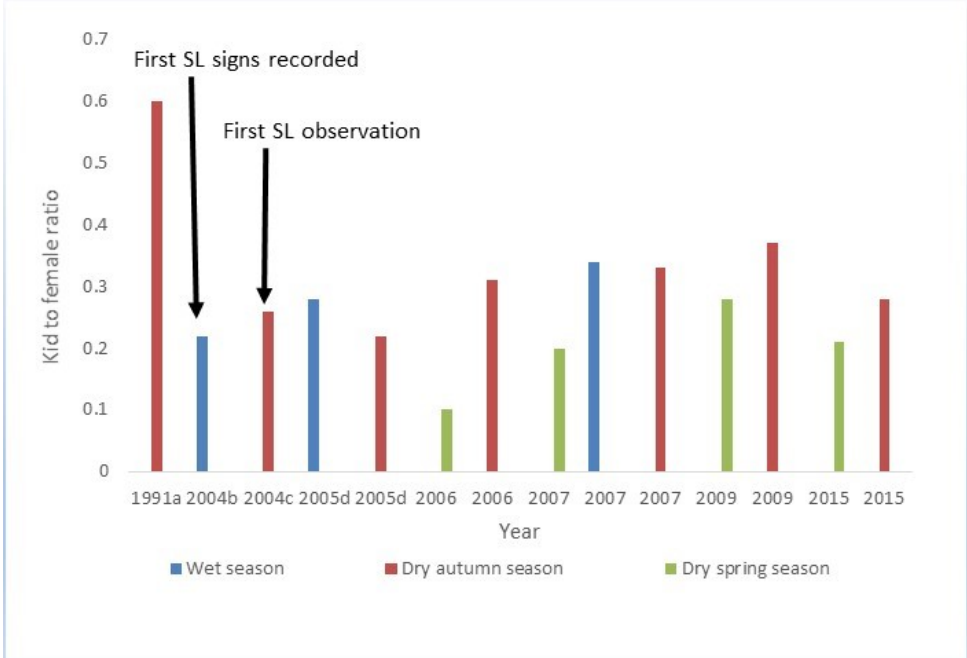

Fig. 5.26 Reproductive rate (kid to female ratio) of Himalayan tahr in SNP in different years and seasons. Letters next to the year mean the source of data: a – Lovari et al. 2009, b – Shrestha 2004 and 2006, c – Ale and Boesi 2005, d – Ale 2007, years not followed by any letter are data from this study.

We analysed mortality rate of Himalayan tahr data from 2006, 2007, 2009 and 2015. Data from 2015 were used only partly, because there were only three records from this year. Number of HT individuals recorded during individual monitoring period fluctuated (Table 5.10) and sometimes happen that more individuals of some category were recorded than were observed

during previous monitoring. It could happen that some individuals were invisible or classified to wrong category, for example, some females could be confused with older yearlings or male individuals were not consistently classified to their classes. However, we believe that more than 90% of TH individuals were counted during each monitoring period.

Table 5.10 Population size of Himalayan tahr in SNP during study period. min – the lowest number of individuals recorded during the year, max - the highest number of individuals recorded during the year, mean – average number of individuals recorded during the year, SD – standard deviation, monitoring period – months when HT individuals were counted.

	min	max	mean	SD	monitoring period
2006	123	176	151.25	24.19	May, Jun, Sep, Oct
2007	78	225	153.43	50.82	Apr, May, Sep, Oct, Nov, Dec
2009	75	134	104.83	23.02	Mar, Apr, May, Oct, Nov, Dec
2015	183	223	203.00	20.00	Apr, Jun, Dec

In each of four study years, number of recorded individuals decreased during dry spring season (March-June), increased in September when new kids were counted in and transition between demographic categories happen. The number decreased again during dry autumn season (Fig. 5.27). The highest mortality rate, calculated over all demographic categories, was during dry autumn season 2007 (Fig. 5.28), when from 225 Himalayan tahr individuals recorded in September 2007 only 117 individuals were recorded in November 2007. Small population size recorded during dry spring season 2009 (Fig. 5.27) probably reflected extremely high mortality in dry autumn season 2007. Population size slowly increased and mortality decreased in 2009 and 2015.

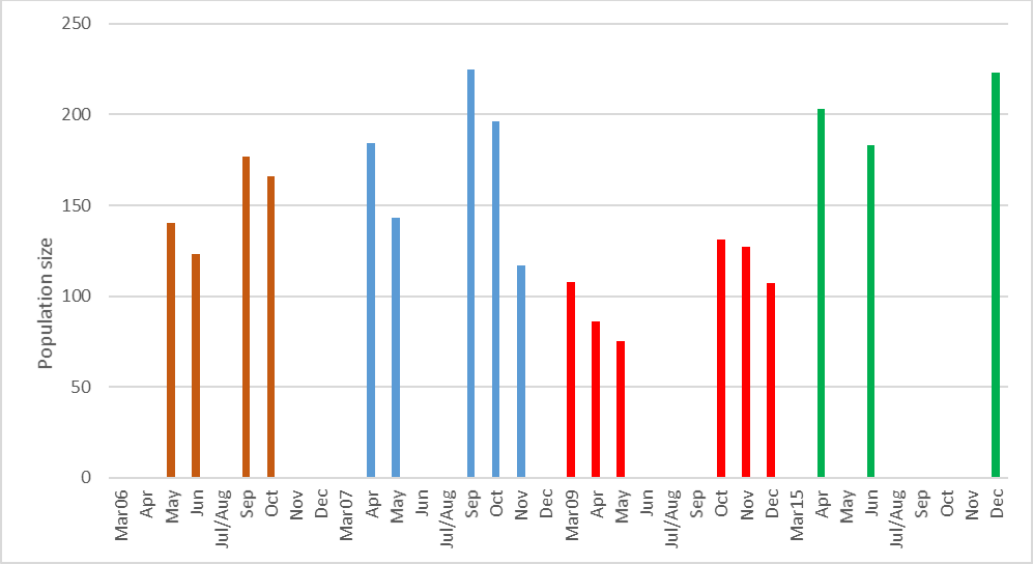

Fig. 5.27 Numbers of Himalayan tahr recorded in SNP during 2006, 2007, 2009 and 2015. Different colours show data from different years: brown – 2006, blue – 2007, red – 2009, green – 2015.

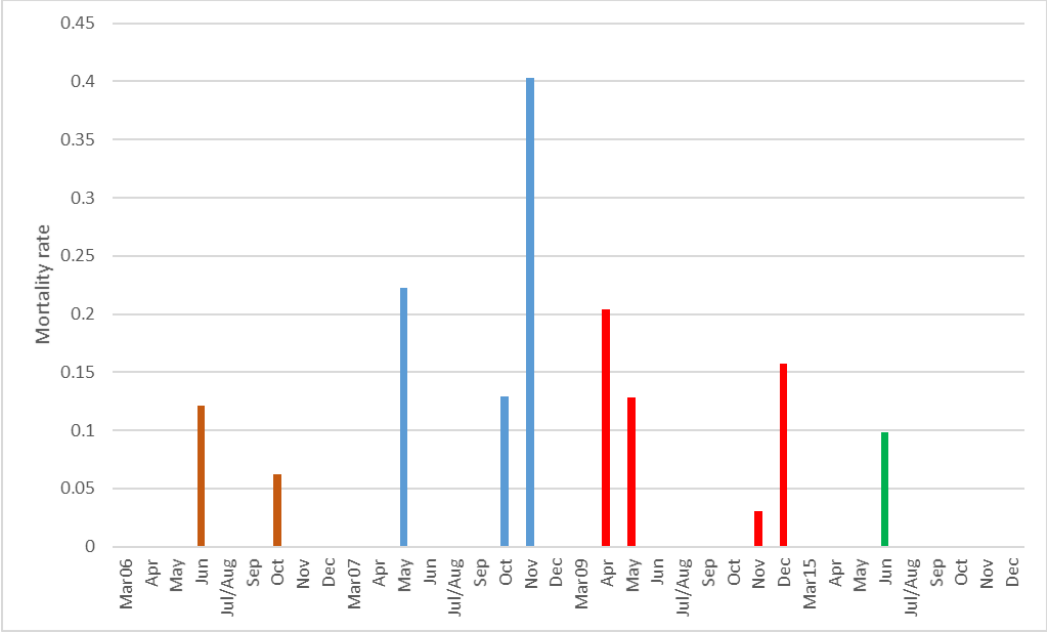

Fig. 5.28 Mortality rate of Himalayan tahr population in years 2006 – 2015. Different colours show data from different years: brown – 2006, blue – 2007, red – 2009, green – 2015. Mortality rate was calculated as 1- Nt /Nt-1 where Nt is the number of individuals of this category in time t and Nt-1 is the number of individuals of the corresponding category in previous time t-1.

The highest mortality rates of all demographic categories were recorded in 2007 (Fig. 5.28 and 5.29). In November 2007 (dry autumn season), mortality rates of kids and yearlings reached 0.7, i.e. 70% of them died between October – November 2007. Also adult individuals died during dry autumn season 2007 but their mortality rates were not so high. The highest mortality rate of females (46%) was recorded in November 2007, whilst the highest mortality rate of

males was recorded in December 2007, when 41% less males than in November 2007 were recorded.

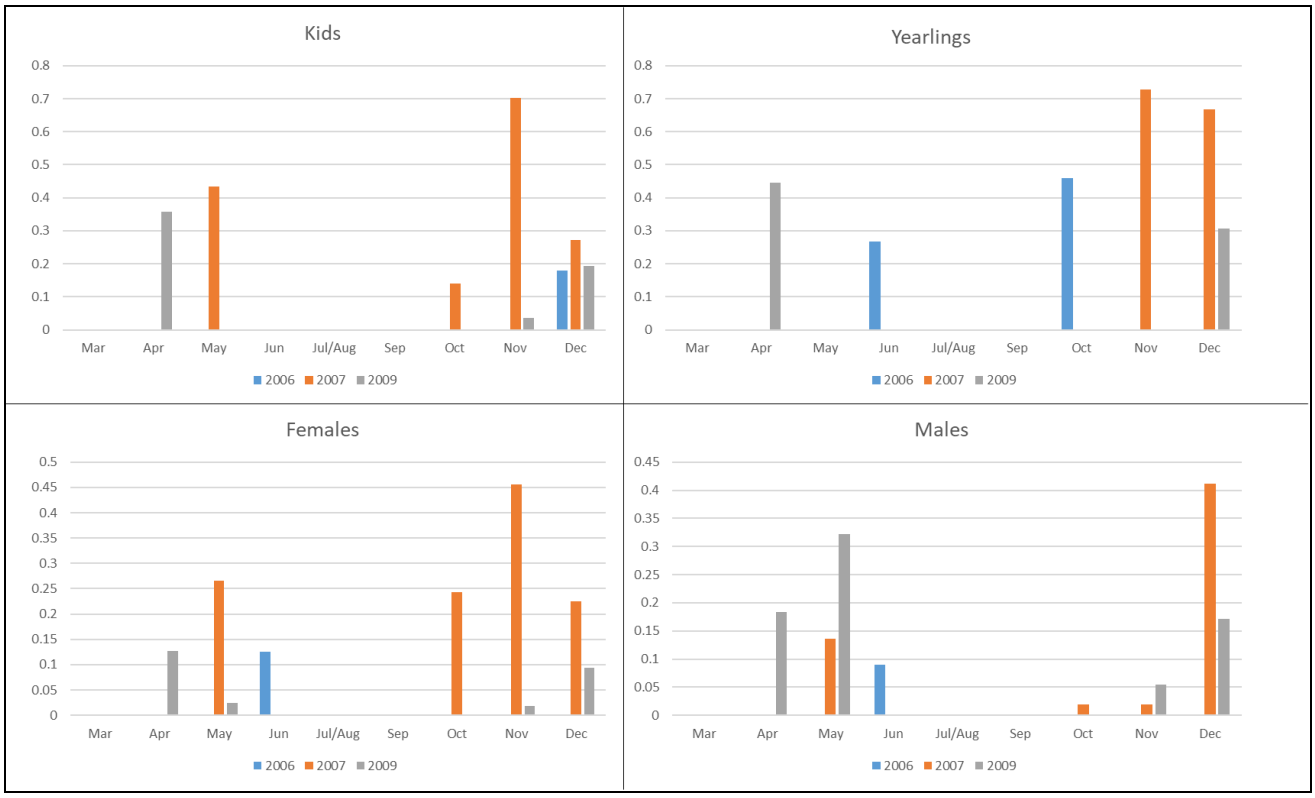

Fig. 5.29 Mortality rates of Himalayan tahr population in years 2006, 2007 and 2009. Mortality rates of four demographic categories (kid, yearling, female and male – sum of all age classes) are shown.

Due to the possible omission of some individuals and uncertainty about the total population size during individual monitoring session we also analysed population structure as a proportion of different demographic categories. We found that proportion of demographic categories fluctuated during the year and there were differences among the years too (Fig. 5.30). Kids created between 3.6% – 0.6% of Himalayan tahr population. Always, the highest portion of kids was recorded in September and October, when new born kids appeared. Their proportion slowly decreased during dry autumn season and was significantly lower in spring. A similar trend, however not so significant, was found for yearlings too. Their proportion in Himalayan tahr population fluctuated between 2.2% recorded in April 2007 and 13.6% in September 2006. Females accounted for approximately half of the Himalayan tahr population (41.2% –53.3%) and proportion of males (all classes together) fluctuated between 19.8% in September 2006, when kids plus yearlings accounted at least one third of Himalayan tahr population, and 43.6% in November 2007.

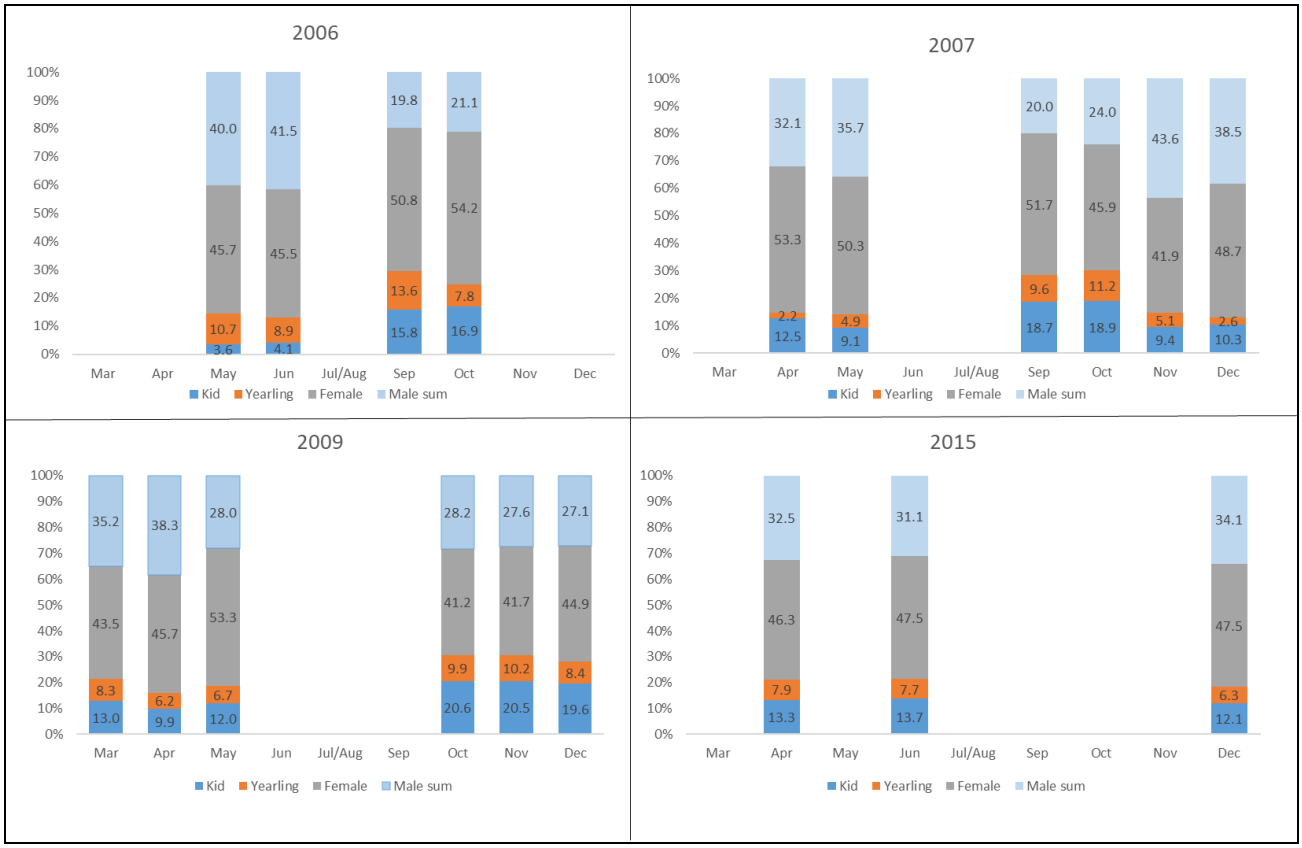

Fig. 5.30 Proportion of different demographic categories in Himalayan tahr population during individual monitoring periods in 2006, 2007, 2009, 2015.

5.4.7 Relation of Snow Leopard and Reproductive Rate of Himalayan Tahr

The reproductive rate of Himalayan tahr was negatively correlated with a density of snow leopard (Fig. 5.31). In 1990, snow leopard was not recorded in SNP and the kid to female ratio was 0.6. Upon the arrival of snow leopard to the area in 2004–2005, when six individuals were recorded (Table 5.7), the ratio of kid to female decreased. During 2009–2015, snow leopard population size decreased whilst the kid to female ratio increased.

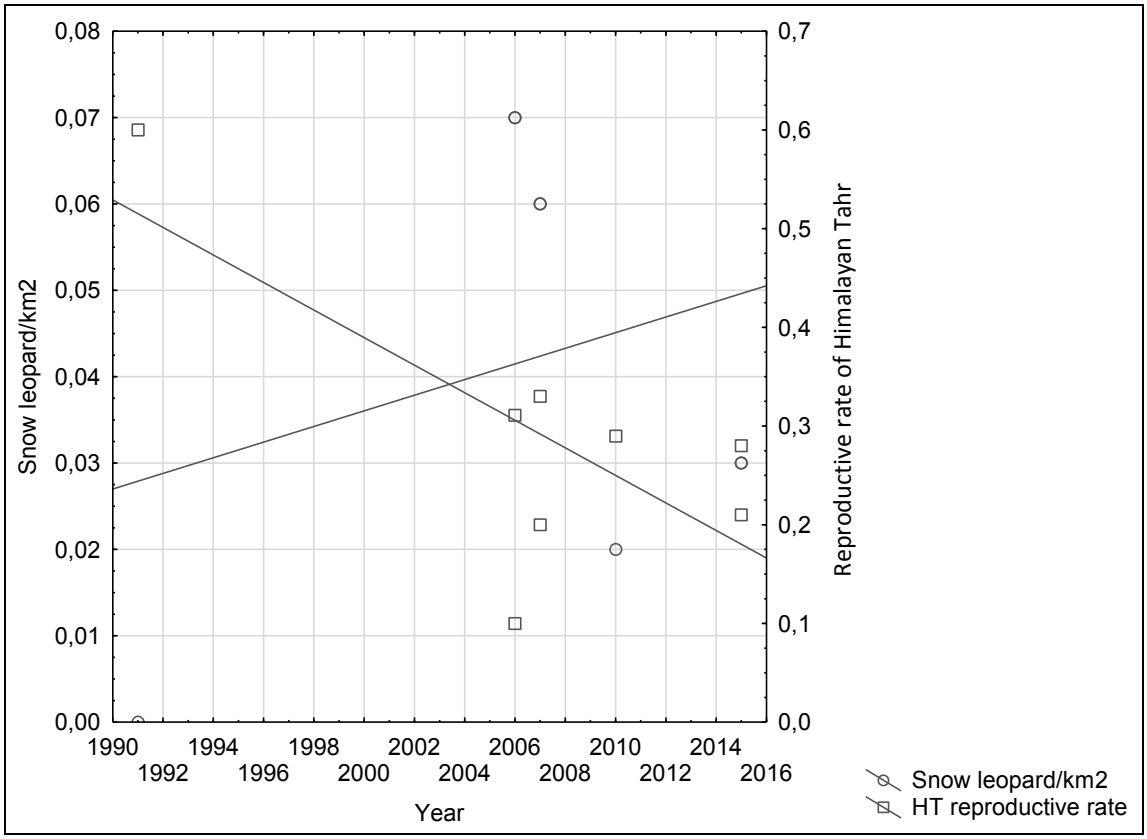

Fig. 5.31 Relation between snow leopard density and reproductive rate in 1990–2016.

5.5 Discussion

5.5.0 Monitored transects

We repeatedly monitored the same transects in SNP but not in LM and UM. This was because we had to establish transects in a lower elevation in LM and UM during dry season 2014 due to high snow layer, and we were not able to use these transects again during wet season 2016, when snow leopards and their prey live in a higher elevation and therefore new transects had to be established. When we compared all transects established in SNP, LM and UM, both in dry and wet seasons, we found significant differences in many transects parameters. However, when we analysed only transects monitored during dry seasons (i.e. 9 LM transects and 17 UM transects monitored during dry seasons 2014 and all SNP transects, repeatedly monitored during several dry seasons) we found no significant differences. Dry season transects from SNP and from LM & UM in ACA partly differed only in proportion of different habitats, topography and ruggedness types. These differences reflected differences in vegetation cover and geomorphology of two study areas. Transects in SNP, where V-shaped rugged valleys, were

also shorter, located in lower elevation and their distances to nearest cliff were significantly larger than transects established in LM and UM, associated with wide U-shaped valleys. Because of the V-shaped valley, transects in SNP were located in lower elevations than transects in LM and UM and their distances to nearest cliff were significantly larger.

In UM and LM, the exposure of valley determines the natural vegetation. It is a transition zone between the moist, southern Himalayan slopes and the high deserts of Tibet (Ale et al. 2014; Shrestha et al. 2018; Shrestha et al. 2020). The cooler, north-facing slopes are densely forested with Himalayan pine (*Pinus wallichiana*), Himalayan fir (*Abies spectabilis*) and Himalayan birch (*Betula utilis*), while dwarf juniper (*Juniperus squamata*), *Caraga*na sp., *Berberis* sp., and some grasses thrive on the drier south-facing slopes, which have higher evaporation rates. Patches of *Pinus excelsa – Betula utilis – Juniperus indica* forests occur along the riverbanks. These alpine valleys fall in the rain-shadow of the Annapurna Mountain Range, with a dry and cold climate. In SNP, the dominant trees are *Pinus wallichiana, Abies spectabilis* and *Juniperus recurve* at altitude ranged of 3,000–3,600 m a.s.l. followed by *Betula utilis, Abies spectabilis* and *Rhododendron barbatum* and *R. arboreum* at altitude of 3,600–3,800 m, *Juniperus sp. and Rhododendron sp.* at altitude of 3,800–4,500 m and birch-rhododendron forests occur at altitude of 3,600–4,200 m a.s.l. mostly, on colder north slopes. Shrubs (*J. squamata* and *Berberis sp.), scrubs (Cotoneaster microphyllus, R. anthopogon, R. lepidotum, R. setosum and R. nivale)* and grass dominate the upper alpine landscape at altitude ranged 3,500–5,000 m a.s.l. (Bufa et al. 1998; Gabarino et al 2014). In the upper part above 3,000m, the rain shadow area of LM and UM receives an average rainfall of less than 260 mm/year (NTNC 2008 a,b) while SNP receive 1,166mm/year at altitude of 3,450 m (Bhattarai and Upadhyay 2013). Therefore, the rangelands of SNP is more productive with grass and shrubs.

Snow leopard and its prey species accommodate all these habitats without significant differences in behaviour. Our transects were placed in the typical snow leopard habitats. Snow leopard mainly prefers rocky and broken terrain, river bluffs, well defined ridgelines, cliffs, gorges, stream beds of gravel and sandbars, vegetation dominated by grasses, shrubs or trees, broken or very broken ruggedness, rolling and flat ruggedness, barren habitats without any vegetation or less grazed areas, forest borders or open shrub land/alpine meadows and sometimes cultivated fields and areas subject to landslides. The species strongly preference slopes in excess of 40° (Jackson and Ahlborn 1988; Jackson 1996; Fox and Chundawat 1997).

5.5.1 Snow Leopard Abundance, Population Size and Density

Our study showed that signs encounter rate (No. of scrapes/km) was positively correlated with snow leopard density obtained by camera trapping. The abundance of scrapes, as well as tracks and scats, may provide a rough index of relative numbers, but counts of scrapes may vary from area to area, because of:

(i) different patterns of terrain and topography,
(ii) local livestock density, which may play a role, because livestock may disturb the scrapes and make then invisible,
(iii) bad weather (rain etc.) can destroy the signs,
(iv) snow leopard itself may behave differentially from place to place (Jackson 1996; Fox and Chundawat 1997; McCarthy 2000) and
(v) density of transect (different total length of transects per 100 km^2).

The ratio of scrapes/km against number of individuals obtained by camera trapping or other methods strongly differs between studies. Fox et al. (1991) reported 2.1 scrapes/km in Ladak (India), with one cat/100 km^2 (ratio = 2.1), while Fox and Chundawat (1997), who collected data in upper Indus Valley, reported 2.5 scrapes/km, with approx. three cats/100 km^2 (ratio = 0.83). Ahlborn and Jackson (1988) reported 11 scrapes/km with of 8–10 cats/100 km^2 (ratio = 1.1–1.37) from Langu valley of west Nepal. In contrast, our study shows 4.5–5 scrapes/km with 6–8 cats/100 km^2 in 2014 in LM and UM (ratio = 0.56–0.83), 2.5–3 scrapes/km with 6 cats/100 km^2 in 2005–2006 (ratio = 0.31–0.5) in SNP and 0.5–1 scrape/km with 3 cats/100 km^2 the same area in 2015 (ratio = 0.17–0.33). Therefore, counts of scrapes as a measure of abundance must take into account the factors (i)–(v) mentioned in the introduction of this chapter. Number of scrapes recorded during dry period 2014 were significantly higher than number of scrapes recorded during wet period 2016. The number of scrapes were higher in dry season than wet season in LM and UM and there are three possible reasons of this observation: i) the transects of dry and wet seasons significantly differed in many of their parameters. During dry season 2014, transects were established in lower elevation than transects monitored during wet season 2016, because in 2014 a high snow cover strongly limited mobility of animals, and observers too, in higher elevation. ii) the likelihood of finding a sign of a snow leopard is greater during dry season than during wet season, when probably snow leopards move to higher elevation sites. These sites are more rugged and get more precipitations, and, therefore, inaccessible to humans in summer. Thus, pressures from

livestock grazing and human presence intensify at lower elevations. Lower abundance of leopard sings in summer than in autumn may be because of snow leopard signs being destroyed by livestock in summer (Ale et al. 2014) and iii) the snow leopard population sizes were different in two monitored periods for each study areas (Table 5.5).

In LM and UM, the photo capture events per 100 trap-nights (RAI) of snow leopard, corresponding with snow leopard population size, showed high inter-annual difference (dry season 2014 and wet season 2016). In SNP, the snow leopard population size has decreased from 2006 to 2015, while its population size has slightly increased in UM from 1990 to 2014 or 2016. During the two years of our study, neither signs, nor photos of snow leopard were observed in Lupra of LM and Thame of SNP. However, snow leopards were recorded in Lupra earlier – in 2010 and 2011 (Ale et al. 2014) and Thame in 2009 (Table 5.1). In Lupra valley, this may be because of increasing pressure of common leopard, which was detected three times here even as high as 4300 m a.s.l. Alternatively increasing pressure of local people collecting *Cordyceps sinensis* in Lupra valley during the wet season, may cause displacement of its prey – blue sheep elsewhere. In Thame valley, the reason may be the decrease of numbers of its other prey, Himalayan tahr from 2006 to 2009 (Table 5.9) or human-snow leopard conflict followed by its retaliatory killing. In SNP also 8 captures of common leopard were detected in forest and shrubland near to forest which could cause interspecific competition with snow leopard, which can affect carnivore abundance (Creel 2001).

The density of snow leopard has slightly increased in UM from 1991 to 2014–2016 as blue sheep population is stable over the time and seems sufficient (above 6.7 individuals/km^2) for the recorded snow leopards (6–8 individuals in the area). In contrast, the snow leopard density has decreased in SNP from 2006 to 2010–2015 as the tahr population has decreased 61 % from 1989 to 2009. In both LM and UM, the snow leopard density has decreased from 2014 to 2016. In 2014, it was due to a hard winter season and heavy snowing. Male and female were active for mating purpose and the habitats were not disturbed by humans. Therefore, camera trap might have captured more snow leopard individuals during winter. In contrast, in 2016, it was summer or raining seasons and the habitats were more disturbed due to yarsagumba (*Cordyceps sinensis)* collection by local people and grazing of yak herds in wet seasons at high altitude which could displaced the blue sheep beyond valleys or the study areas. Therefore, less snow leopard individuals were captured in 2016. Because of small effective sample sizes and animals move possibly beyond the study area these estimates should be viewed with caution in cases of LM and UM.

5.5.2 Population Dynamics of Blue Sheep in Lower Mustang and Upper Manang

Blue sheep density was higher in UM than in LM. It could be because of more overgrazed and degraded land in LM. Based on our field experience and NTNC (2008a, b), LM is more barren and unproductive area than of UM. Moreover, livestock pressure is also low in UM. The reproductive rate of blue sheep (0.67) is satisfactory, which indicates that the population is increasing. However, in UM, the blue sheep density was more or less constant during 1990–2016 (Fig. 4.5). The plausible reason for the lack of changes in population size in this area could be: (i) slight increase of snow leopard density, one or two more individuals than was recorded in 1990 and (ii) environmental stochasticity, as we observed the heavy snowing and avalanches in 2014 and 2015 killed ~ 50 adult male blue sheep in UM. In LM, the number of blue sheep was 30% lower in wet season 2016 than in dry season 2014 although the predator pressure was lower as the number of snow leopard individuals were lower in 2016. The reasons of the lower number of blue sheep in 2016 might be i) from disturbance factors such as (yarsagumba (Cordyceps sinensis) collection by local people and grazing of yak herds in wet seasons at high altitude which could displaced the blue sheep beyond valleys or the study areas and ii) possibility of missing some adult male blue sheep during counting because they were segregated from female herds during wet seasons and wandered widely at high altitude which could make difficult to find them in foggy weather. Therefore, male to female ratio was 0.62:1 in LM in 2016. These both reasons are applied in case of UM also which caused high fluctuation in number between valleys. In Proper Manang, the yak herds were grazed during wet seasons but not in Yak Kharka.

5.5.3 Population Dynamics of Himalayan Tahr in the Sagarmatha National Park

Himalayan tahr population decreased from 1989 to 2009 by 63% (from 350 to 134 individuals, Fig. 4.24) and after that the population increased by about 70% to 223 individuals in 2015. This is, however, still less than 350 individuals in 1989 reported by Lovari (1992). Unlike blue sheep number fluctuation between valleys during dry and wet season, tahr numbers have more or less constant between valleys in both seasons in 2007. Namche and Phortse valleys are separated by deep gorge of two rivers, which can be a barrier for tahr to move between valleys. People do not collect *Cordyceps* in SNP during wet seasons.

The results showed the inverse proportion has occurred between Himalayan tahr number and snow leopard sign in the four valleys of SNP (Table 5.9 and Fig 5.19). For example, Namche and Thame with its higher density of Himalayan tahr revealed a lower snow leopard sign. A similar finding was reported by Fox and Jackson (2002) on inverse relationship between

snow leopard signs density and blue sheep in trans-Himalayan Nepal (blue sheep 2–4/km^2 and 2.8 snow leopard signs/100 km of sign transect) and in Bhutan (blue sheep 4–6/km^2 and 1.2 snow leopard signs/100 km). Ibex *Capra ibex* density does not appear to be a particularly good predictor of snow leopard sign density in Mongolia (McCarthy 2000; Ale 2005). It has been shown that density of prey only may not predict predator abundance but also biomass of all available prey, including livestock and small mammals (Ale 2005), and other factors also such human-snow leopard conflict level (e.g., retaliatory killing) and disturbance factors caused by human (roads, trekking, sound, light, etc) may prove a better predictor.

Overall, reproductive rate of tahr was low (below 0.3). In contrast, Schaller (1973) reports a kid to female ratio was 0.56 in Kang Chu, eastern Nepal, where tahr is hunted, and 0.57 in the Annapurna regions of western Nepal (Gurung 1995). The low reproductive rate of tahr in Sagarmatha may be due to (i) predation, (ii) disease or (iii) over grazing by livestock.

The recent study by Shrestha et al. (2018) reports that Himalayan tahr is a dominant prey of snow leopard in SNP. Our study revealed that the reproductive rate of Himalayan tahr was negatively correlated with the density of snow leopard (Fig 5.31). In 2004–2006, i.e. time of snow leopard re-occurrence, when six individuals were recorded in the area, the predation rate on Himalayan tahr was probably high because the tahr lacked anti-predation behaviour. Therefore, the mortality rate of tahr was high in 2007 and this trend could continue in 2008 too. Therefore the Himalayan tahr population size was low in 2009 and subsequently the snow leopard population decreased to only two individuals in 2010. On the other hand, tahr moves down to the forested area during winter season (January-February) and camera traps placed near to such area captured 8 capture events of common leopard in Namche and Phortse. Therefore, in winter, predator impact on tahr could be high due to pressure of two predator species. In addition, diseases, e.g. brucellosis, or other infections, may affect the fertility of females and neonatal-juvenile mortality in ruminants but Lovari et al. (2009) did not found any pathogens in Himalayan tahr blood samples.

The competition for forage between tahr and livestock could cause decrease of tahr population. Shrestha et al. (2012) showed that there was a large overlap in the diets of tahr and livestock expressed by Morisita index ($C_H = 0.83$). However, this study was carried out in monsoon season, when net primary productivity is high and forage quality very good. During the summer season, tahr is more agile than livestock, able to reach the vegetation growing in steep and rocky areas and therefore spatially separated from livestock (Shrestha et al. 2012). Therefore, strong competition for food is unlikely during summer. However, the forage quality is very low in winter season. In winter, herders get their livestock down to village and near to

forest. Similarly, tahr moves down to forested areas during January-February. In shrub and forest areas, both yak and tahr must eat unpalatable forage, including shrubs and tree bark and dietary overlap might be 100% (Shrestha et al. 2012). In Namche areas, people has left the traditional rotation grazing system livestock and graze freely throughout the year. Therefore, in winter livestock and tahr could compete for food in SNP. Most livestock removed large amounts of forage from the pastures (Bagchi et al. 2004), otherwise available to native prey.

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Paper IV

Implications of landscape genetics and connectivity of snow leopard in the Nepalese Himalayas for its conservation

Bikram Shrestha and Pavel Kindlmann

(see attached published paper)

Paper V

Hair identification key of Himalaya mammals of Nepal as a tool to study food habits of snow leopard

Bikram Shrestha and Pavel Kindlmann

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ABSTRACT

Large carnivores like snow leopard are solitary and elusive species which makes observing their hunting and feeding behaviour difficult and moreover small preys are completely consumed and it is not easy to detect their kill site (Oli 1993). However, prey hairs are not damaged in a stomach during the process of mastication and digestion. The prey hairs in predator's faeces are therefore a reliable and important tool to study food habits of carnivores. Number of scientific publications focusing on microstructure animals' hairs is available from the end of the 19th century. However, only a few publications (Oli 1993; Khatoon 2010; Anwar et al. 2012) dealing with hair-microstructure of animals are available from the snow leopard habitat and detailed examinations as micro-measurements and microscopic hair characteristics are still lacking. The aim of this chapter is to provide such a detailed microstructure characteristics and micrometres measurements from the hairs of Himalayan mammals sampled within the snow leopard habitats in Nepal. Moreover, digital photographs reference key of the medulla, cuticle (scales) and cross-sections of guard hairs with the description of hair characteristics are provided as a tool for hair identification.

4.1 Introduction

The first historical reference of hair identification can be traced back to Brewster (1837) who described the micrometres of microscopic objects including mole's hair and Quekett (1844) who studied on the structure of bat's hair. It was followed by Browne (1860: in Brothwell and Spearman 1963) and Pruner-Bey (1877) who examined mummy hair from South America and Egyptian-Peruvian respectively, and McMurtrie (1886) who published a scientific paper on mammalian hair. Thereafter, considerable scientific publications on microstructure animals' hairs are available from the end of the 19th century (Hausman 1920, 1924 and 1930; Koppikar and Sabnis 1975; De Boom and Dreyer 1953; Day 1966; Dreyer 1966; Brunner and Coman

1974; Stuart 1976; Perrin and Campbell 1979; Keogh 1983; Teerink 2003; Debelica 2009; Cornally 2016) for the purpose of wildlife biology, veterinary, forensic and medical studies such as taxonomy, carnivore food habits and epidemiological studies. However, only a few publications (Oli 1993; Khatoon 2010; Anwar et al. 2012) on hair-microstructure of animals are currently available from the snow leopard habitat and detailed examinations on micro-measurements and microscopic hair characteristics are still lacking.

These examinations might elucidate population dynamics from the view of trophic ecology. Predator hunting behaviour and prey selection affect the population dynamics of their prey, which in turn affects the population dynamics of these large carnivores. Therefore, our understanding of their trophic ecology and foraging strategies is important for predicting their population dynamics and consequently for developing effective conservation programs (Shrestha et al. 2018). However, large carnivores like snow leopard are solitary and elusive species which makes it difficult to observe their hunting and feeding behaviour. Moreover, small preys are completely consumed and it is not easy to detect their kill site (Oli 1993). Only hairs are not damaged in the stomach during the process of mastication and digestion because the high content of cysteine-containing keratin and dead keratinocytes. These components make the hairs resistant to post-mortem changes and chemical decomposition (Harkey 1993; Knecht 2012). and so hairs retain many identifiable features (Oli 1993).

Each hair consists of hair root located below the surface of the skin and shaft located above the skin's surface. The hair root is embedded in the dermis of the skin in the hair bulb. The bulb surrounds the hair papilla which contains blood vessels and nerves nourishing the root, adding new cells while forming the shaft of the hair which extends above the skin. The cells of the shaft become keratinized, hard and soon die, so that the hair protruding above the skin is a dead structure. Typically, the hair shaft consists of three layers: an external cuticle made up of overlapping microscopic scales, middle cortex containing shrivelled cells and pigments, and inner medulla built from many shrunken and variously disposed cells or champers. The mammalian coat is composed of guard hair or over hair which is long, stiff and also responsible for normal texture of the body and fur or under hair which is thin, soft and usually undulating in appearance and has less taxonomic value (Debelica 2009; Chakraborty 2010).

This chapter provides a detailed microstructure characteristics and micrometre measurements of the hair shaft parts (medulla and cuticle) of Himalayan mammals sampled within snow leopard habitats in Nepal. Moreover, digital photographs reference key of the

medulla, cuticle (scales) and cross-sections of guard hairs with the description of hair characteristics are provided as a tool for hair identification.

4.2 Materials and Methods

The identification of hair samples required a reference collection of hair samples, microscopical hair slide preparation and an identification key.

4.2.1 Reference Hair Collection

Reference hair samples (guard hairs) were collected from all potential mammalian prey species (wild and domestic animals) of snow leopard from three study areas, mainly from Sagarmatha National Park and some samples from Annapurna Conservation Area (Lower Mustang and Upper Mustang) in the course of snow leopard's diet study in 2006 and 2007 (Shrestha 2008; Shrestha et al. 2012), and 2014–2016 (Shrestha et al. 2018) respectively. Entire hair tufts from different body parts of live animals and available carcasses were collected from hunting sites. The reference hairs of 10 wild species (Himlayan tahr *Hemitragus jemlahicus*, blue sheep *Pseudois nayaur*, musk deer *Moschus chrysogaster*, snow leopard *Panther uncia*, red fox *Vulpes vulpes*, woolly hare *Lepus sp*, Mountain weasel *Mustela sp*, shrew *Soricidae*, rat *Rattus sp*, pika *Ochotona*) and 5 domestic animals (Yak *Bos grunniens*, dog *canin*es, cow *Bos sp*, horse *Equus sp*, mountain goat *Capra sp*) were collected.

4.2.2 Microscopical Hair Slide Preparation

Each hair sample was washed in tap water for several times to remove dirt and was dried on a clean watch glass. Then the samples were examined macroscopically, and the colour and texture recorded. The sample was put into 30% hydrogen peroxide overnight prior to microscopic examination. The hairs were examined following Brunner and Coman (1974), Keogh (1983), Oli (1993), Rijal et al. (2004) and Shrestha et al. (2012). A technique for studying microscopic hair features was used to identify following characteristics: A) cuticular scale cast or impression, B) whole mounts and examination of the medulla and C) cross-section.

A. cuticular scale cast or impression: A thin layer of nitrocellulose lacquer (white nail polish) was applied on the surface of microscope slides and the hair samples were placed horizontally on them. As the lacquer dried, the surface of each hair formed an impression that the hair was peeled off when viewed under a microscope. The characteristic of the cuticular scales were noted along the shaft from the basal to the tip for each hair. As the scale pattern varies along the length of the hair, it has been described at the base, the mid-region of the shaft and the tip-region of the hair. The cuticular characteristics were examined on different forms of three parameters; scale margin, scale distance and scale pattern.

(i) Forms of the scale margin

a. smooth margin: margin of the scales is smooth without ripples, indentations or serrations

b. crenate margin: scale margin has shallow and pointed indentations

c. ripple margin: the indentations are relatively deeper in comparison to crenate pattern and are rounded

d. scalloped margin: series of curves at the margin having rounded peaks and pointed troughs

e. dentate margin: the scales of margins show tooth like projections

(ii) scale distance: the distance apart of the scale margins were visualized arbitrarily from the diagrams and categorized into three form; distant, near and close.

(iii) scale pattern

a. regular wave: scales are on overlapping and wavy, continuous with the length of wave

b. irregular wave: scales are wavy in appearance and continuous but the height of the crests and the depth of the troughs varies along the wave

c. diamond petal: scales are overlapping and the peak of the lower scale usually touches the trough of the upper scale giving a diamond pattern like appearance.

d. narrow diamond petal: modified form of diamond petal type with lesser width of the scales and greater length.

e. single chevron: scales are non-overlapping with wave like structure with either the crests or the troughs, but not both, have narrow V-shaped structure.

f. broad petal: scales are overlapping with broad and have irregular size and shape.

g. flattened irregular mosaic: scales of dissimilar size and shapes, and scales are wider than their length.

h. transitional: the scale pattern changes as one progress from the base to tip of the hair and the point where one pattern changes to another, shows transitional pattern.
i. streaked: irregular wave but interrupted at regular interval by longitudinally running columns of scales with steeply margins.

B) whole mounts and examination of the medulla: each bleached hair sample was chopped into small pieces about a length of the slide and dipped in xylene for 3–4 hours. Then the hair sample was wet mounted in D.P.X and the cortex and medulla of the hairs viewed under microscope and the details recorded. The micrometre measurement of both the cortex and medulla were recorded at ten intervals along the shaft of each hair. These measurements were then converted to millimetres and the mean calculated. Similarly, the average diameter of the medulla was determined. In addition, medullar indices and their averages were calculated. The above calculations were made only for medullated hair. The medullary index (MI) was calculated using the formula

$$\text{MI} = \frac{\text{Width of medulla}}{\text{Width of cortex}}$$

The characteristics of medulla types were classified as follows:
a. fragmented medulla: medulla is interrupted along the length of the hair by short sections of cortical material.
b. continuous medulla: medulla without fragmented.
c. wide medulla lattice: medulla is wide and fills most of the hairs and each grid of lattice is like pentagonal or hexagonal in shape.
d. uniserial ladder: medulla has a single row of air space along the length which are round, cup, angular or flattened in shape.
e. double or multiserial ladder: medulla with two or more distinct rows of mostly uniform air spaces.
g. simple medulla: medulla has no specific pattern in it and has amorphous appearance.
h. scalloped medulla margin: edge of medulla looks like semicircle along the length of medulla.

C) cross-section: a tuft of hairs was inserted into a straw and then molten (wax liquefied by heat) was sucked into the straw. Once the hairs were solidified, the straw was cut and opened, then core and embedded hair were removed and cross section was obtained by transversal cut with a razor blade. The sections were treated with xylene to remove the wax and viewed under

a microscope. The cross-section of hairs was classified into different types based on medulla shape (circle, oval, biconcave, dumb-bell) and medulla size (wide, medium, narrow).

4.2.3 Hair Identification Key

First, macroscopic view of hair samples was noted. Second, microphotographs of representative scale casts, medulla and cross-sections along the length of the hairs of each species were taken at 200X and 400X magnification using a light microscope and the photographing program BEL Eurisko^{TM}. Several photographs were taken from the base towards the tip of the hair so that the change in cuticle pattern along the hair was clearly visible. Afterwards, a detail characteristic of the microphotographs was also noted.

4.2.4 Identification of Unknow Hairs (of Consumed Prey) in Snow leopard Scat

Each scat should soak overnight in liquid dettol mixed with water and then it was washed carefully over a sieve with 1mm mesh. Microscope slides preparation of the hair were used to identify the species of prey by using the reference key and the microphotographs of the hairs of potential prey species.

4.2 Results

4.2.1 Macroscopic view

Overall thickness, colour pattern and shaft length of the hair samples were described in Table 4.1. Overall shaft thickness and the length of hair to be useful to separate large to small prey species hair whereas the former has thicker and longer hairs. Colour patterns and texture of the hairs were also important to distinguish the species.

Table 4.1 Macroscopic characteristics of hairs

Species	Macroscopic view of hairs	Shaft length (cm)
Himalayan tahr	Thick shaft, proximal: white; middle: grey/copper; distal: grey/black, straight or wavy	5.5–11.1
Blue sheep	Thick shaft, white, black and grey, brittle hair, wavy	3–7.5
Musk deer	Thick shaft, proximal: white; middle: light black; distal: yellow; tip: black, white part almost straight, rest is wavy, brittle hair	1.7–6.2
Snow leopard	Thin shaft, black and white, mostly wavy ad few white and straight	5–11.5
Red fox	Moderate thick shaft, most hair respectively (proximal to distal) have white, red/dark brown, white/light brown and black. Few have proximal white and rest black	5.5–6.9
Yak	Thick shaft, black hair: thick, mostly wavy and few straight; light golden hair: comparatively thin and wavy	10–30
Dog	Moderate thick shaft, black, white, curved, wavy	4–6.5
Cow	Moderate thick shaft, black, straight	3.8–5.1
Horse	Thin shaft, black, white or red brown, straight with slightly curved	3–8
Goat	Thick shaft, white and few browns, slightly wavy	5.8–9
Woolly hare	Very thin shaft, proximal to middle: light black and extreme thin; Middle: black and moderate thick; distal: white and thick, tip: black	2.8–3.6
Mountain weasel	Moderate thick shaft, proximal: dark grey and white; tip: yellowish to light brown	2
Shrew	Very thin shaft, proximal and distal: yellow; middle: ash, slightly wavy	0.8
Rat	Thin shaft, proximal to middle: light black; middle to distal: black, mostly straight, few curve	1.9–2.8
Pika	Very thin shaft, root: dark ash; middle: white; tip: black, slightly curved	1.5–2

Proximal end – hair root or end of the hair closest to the body

Distal end – hair tip or end of the hair furthest from the body

4.2.2 Microscopic view

Micrometre and microscopic diagnostic characteristics of guard hair of all potential mammalian prey species of snow leopard inhabiting Sagarmatha National Park and Annapurna Conservation Area (Lower Mustang and Upper Manang) were examined and explained in Table 4.2 and Table 4.3.

Table 4.2 Micrometre measurements of cortex, medulla and calculation of medullary index of guard hairs of mammalian prey species of snow leopard in Nepal

Species	Cortex diameter (μ)	Medulla diameter (μ)	Medullary index (average)

Himalayan tahr	65–175	30–155	0.717
Blue sheep	220–250	110–240	0.789
Musk deer	150–335	140–325	0.943
Red fox	60–85	40–70	0.788
Snow Leopard	40 –70	15–30	0.406
Yak	70–110	5–20	0.119
Dog	25–75	5–25	0.319
Cow	45–60	15–60	0.431
Horse	30–55	10–30	0.531
Goat	150–215	85–175	0.722
Woolly hare	25–65	15–65	0.763
Mountain Weasel	50–87	36.5–60	0.735
Shrew	12.5–22.5	12.5–17.5	0.802
Rat	40–110	30–100	0.871
Pika	10–55	5–52.5	0.88

Table 4.3 Microscopic diagnostic characteristics of guard hairs of mammalian prey species of snow leopard in Nepal. Terminology of the characteristics were described in methods

Species	Scale margin	Scale distance	Scale pattern	Medulla type	Cross-section	Figure
Himalayan tahr	Smooth or slightly scalloped	Distant	Regular wave	Continuous, simple straight or very slightly scalloped margin	Circular, wide and porous medulla	4.1 a – d
Blue sheep	Smooth	Distant	Broad petal	Wide medulla lattice	Oval or circular, wide medulla	4.2 a – e
Musk deer	Smooth	Distant	Flattened irregular mosaic	Wide medulla lattice	Circular, wide and large porous medulla	4.3 a – d
Red fox	Smooth and dentate	Distant	Diamond petal	Continuous, regular fissure or fringed margin	Circular o oval, wide medulla	4.4 a – e
Snow Leopard	Rippled or scalloped	Near	Irregular wave	Continuous, simple, slightly scalloped margin	Circular, medium size medulla	4.5 a – c
Yak	Crenate or rippled	Near	Irregular wave	Fragmented, thin, heavily pigmented	Oval, narrow or absent medulla	4.6 a – c
Dog	Smooth or slightly crenate	Distant	Regular wave	Continuous, simple or uniserial ladder	Circular, medium size medulla	4.7 a – f
Cow	Smooth or slightly crenate	Distant	Regular or irregular wave	Continuous, simple, straight margin	Circular or oval, medium size medulla	4.8 a – d
Horse	Crenate	Close	Regular or irregular wave	Continuous, simple, straight margin	Oval, medium size medulla	4.9 a – c
Goat	Rippled and Crenate	Close	Irregular wave	Continuous and simple or slightly fringe	Circular or oval, wide medulla	4.10 a – e
Woolly hare	Smooth and dentate	Distant	Regular wave, chevron	Continuous, scalloped margin	oval or biconcave, wide medulla	4.11 a – g
Mountain Weasel	Rippled	Close	Irregular wave	Unbroken vacuolated at middle shaft, Irregular uniserial ladder and scalloped margin at distal	Circular or oval, wide medulla	4.12 a –d

Shrew	Smooth and dentate	Distant	Diamond petal and transitional	Elongated uniserial ladder	Circular or biconcave, wide medulla	4.13 a – f
Rat	dentate and slightly crenate	Close	Diamond petal and Irregular wave	Wide medulla lattice, elongated fringed, scalloped margin	Circular, conccavo–convex, dumb-bell medulla, wide medulla	4.14 a – f
Pika	dentate and smooth	Close	narrow diamond petal and regular wave with slightly streaked	Irregular stacked, irregular double bands or ladders	Circular, wide medulla	4.15 a – d

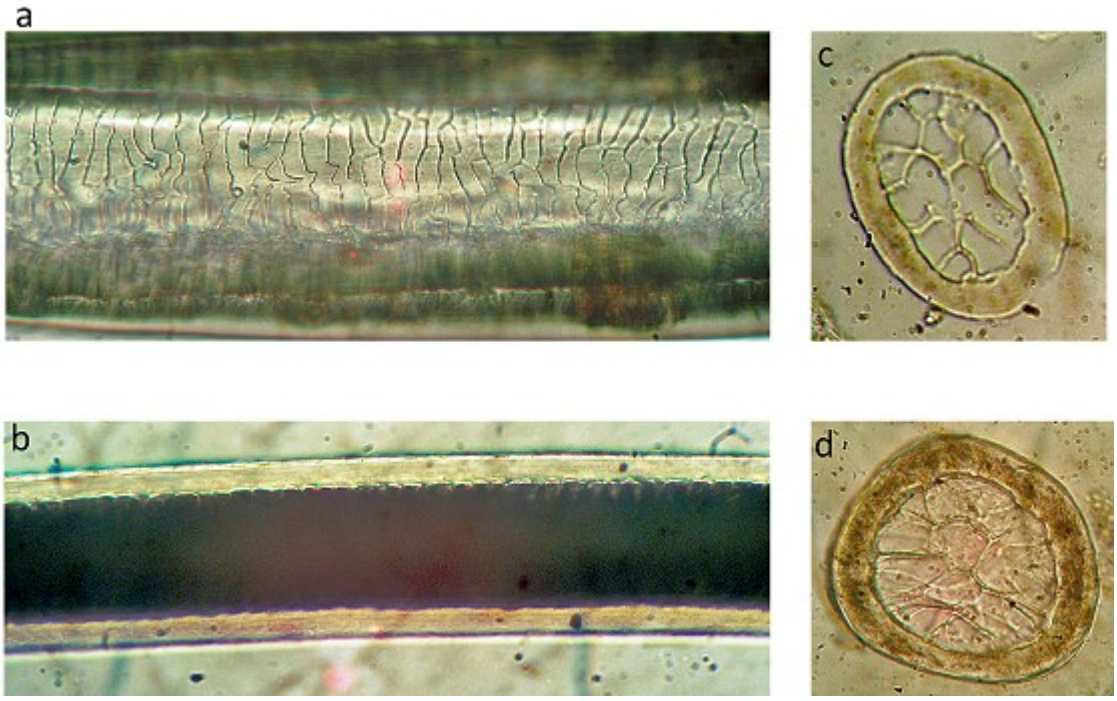


Fig. 4.1 Microphotographs (all 400X) of guard hair of Himalayan tahr. a, predominant scale; b, predominant whole mount; c and d, cross-section

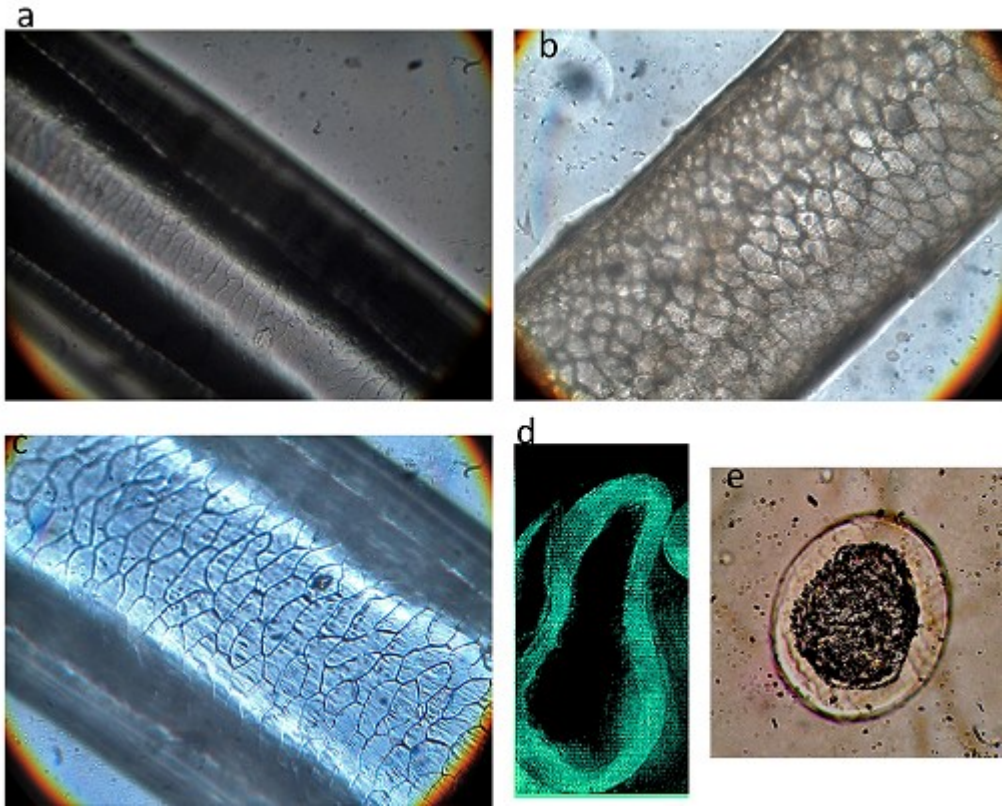


Fig 4.2 Microphotographs (all 400X) of guard hair of blue sheep. a, scale at tip; b, whole mount; c, predominant scale pattern; d and e, cross-section

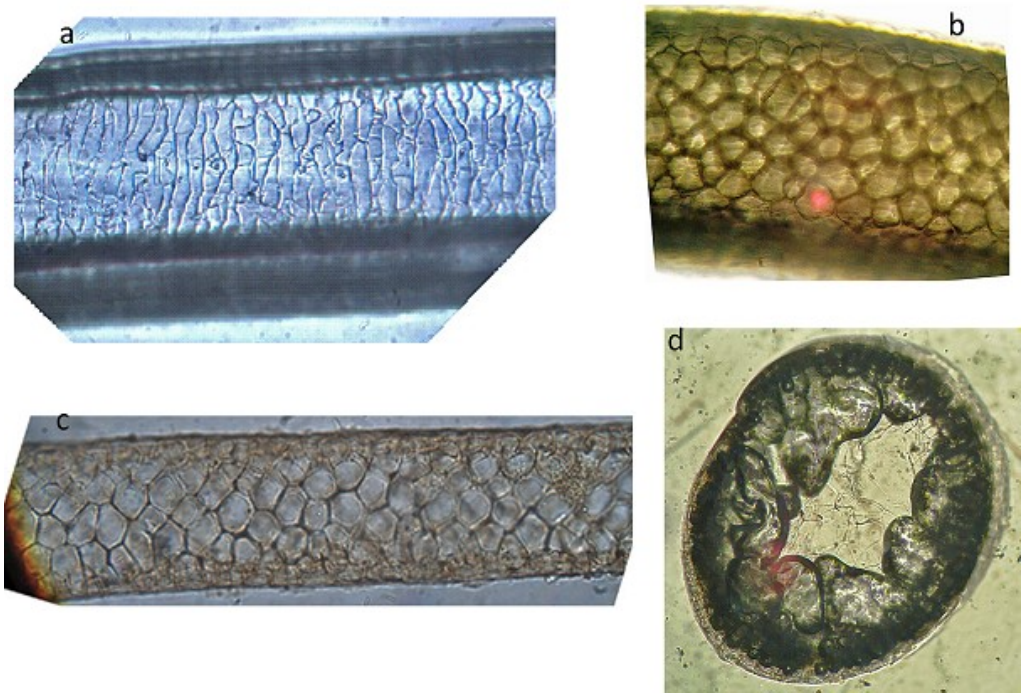


Fig. 4.3 Microphotographs (all 400X) of guard hair of musk deer. a, predominant scale pattern; b and c, whole mount; d, cross-section

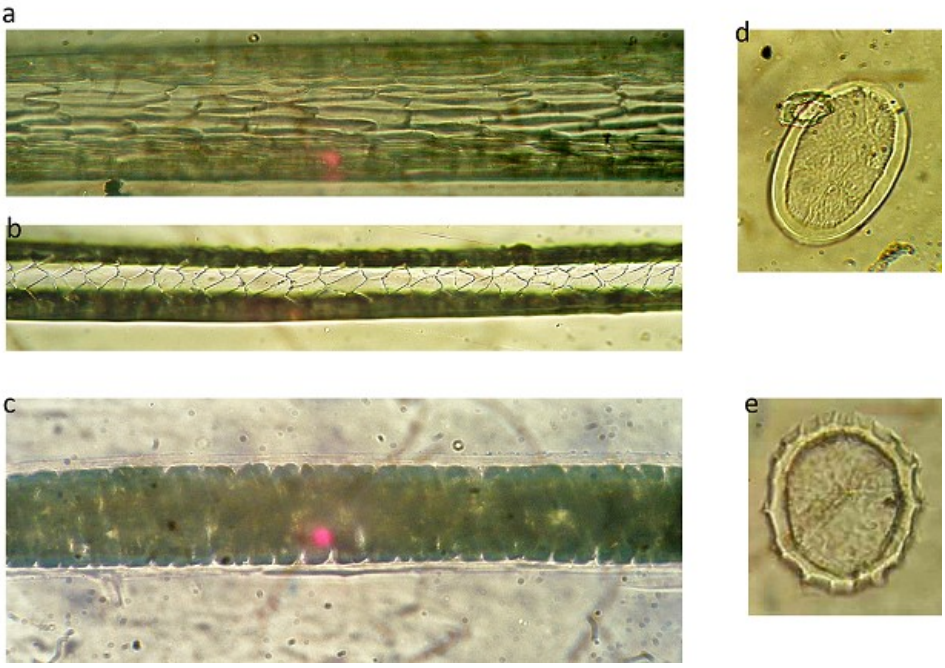
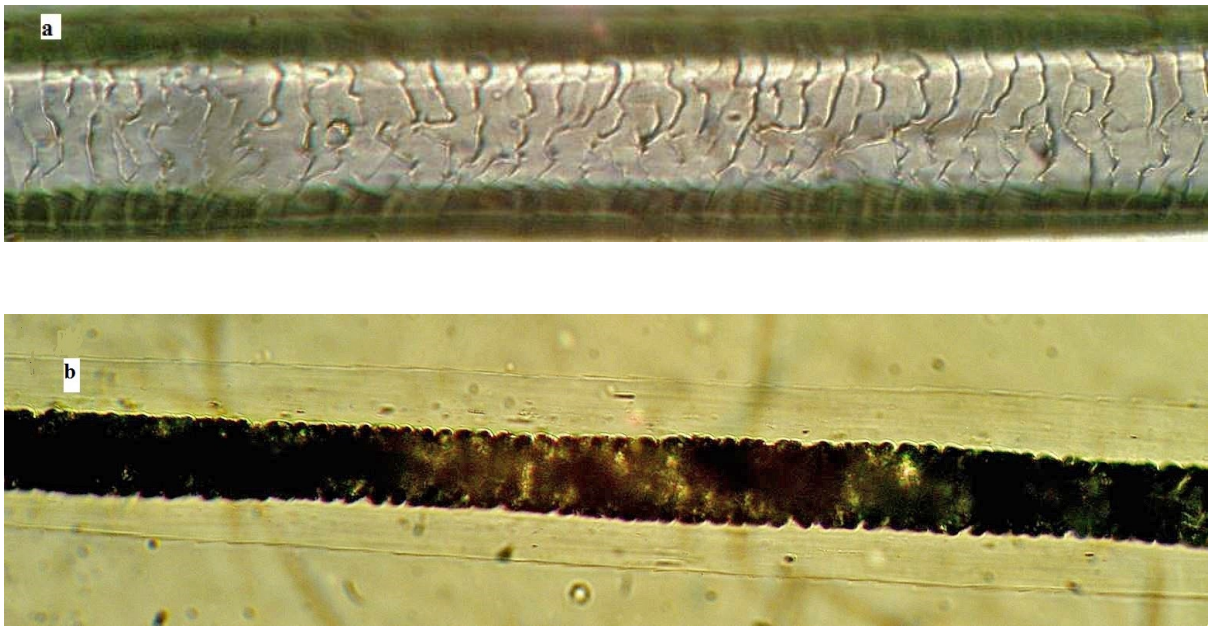


Fig. 4.4 Microphotographs (all 400X except b 200X) of guard hair of red fox. a, scale pattern at proximal; b, scale pattern at middle; c, whole mount; d and e, cross-section



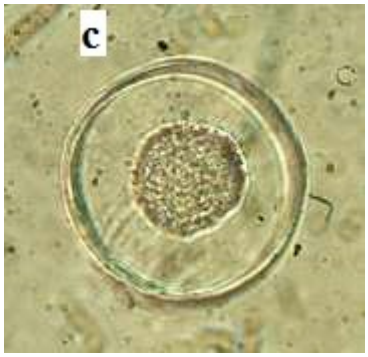


Fig. 4.5 Microphotographs (all 400X) of guard hair of snow leopard. a, predominant scale pattern; b, whole mount; c, cross-section

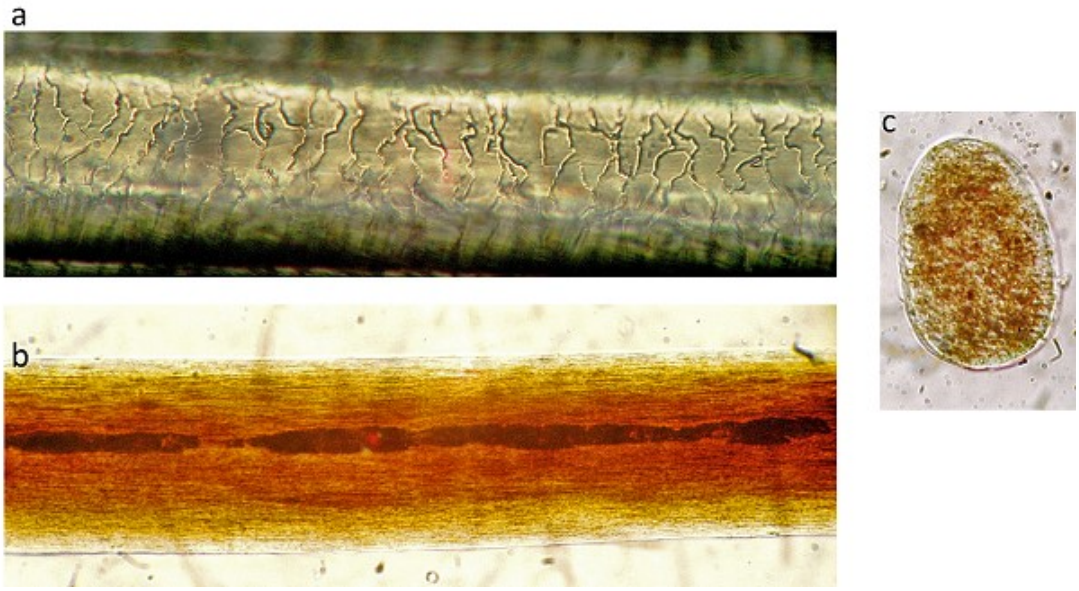


Fig. 4.6 Microphotographs (all 400X) of guard hair of yak. a, predominant scale pattern; b, whole mount; c, cross-section

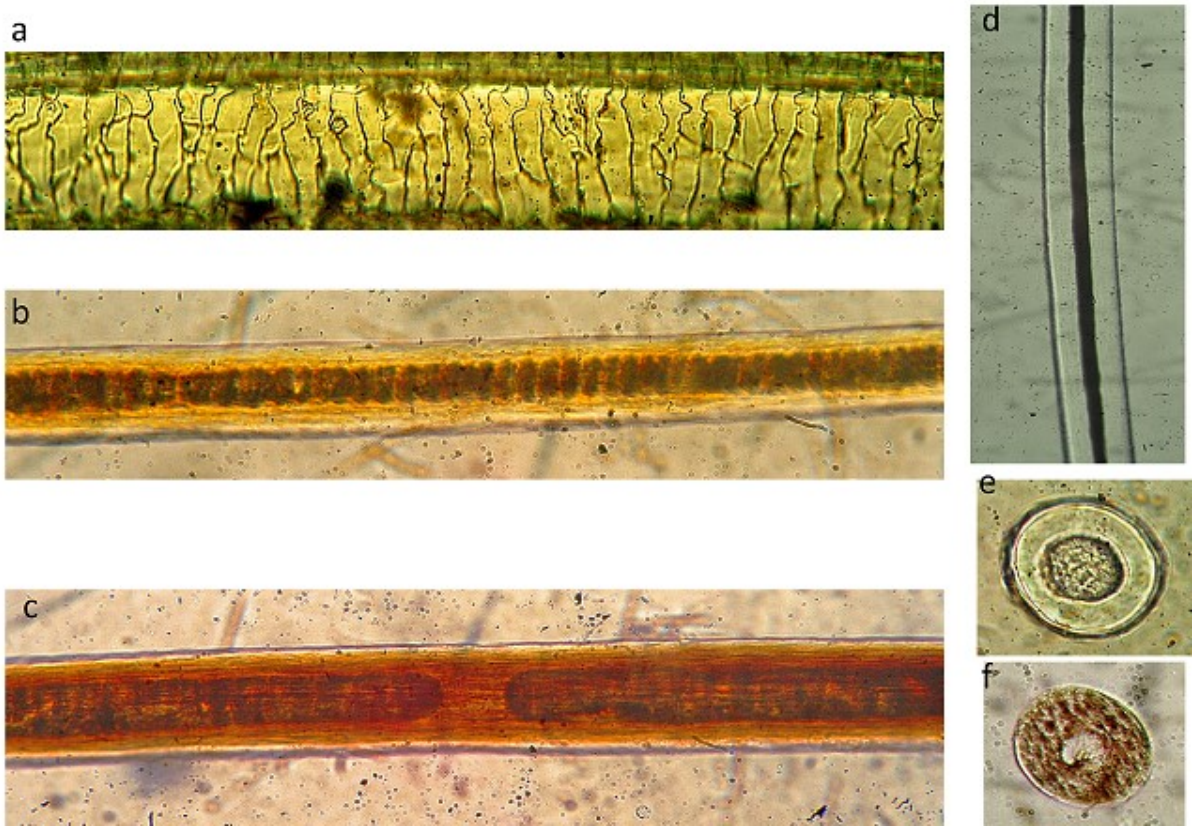


Fig. 4.7 Microphotographs (all 400X) of guard hair of dog. a, predominant scale pattern; b, c, whole mount of tibetan black dog; d, whole mount of small size white dog; e and f, cross-section

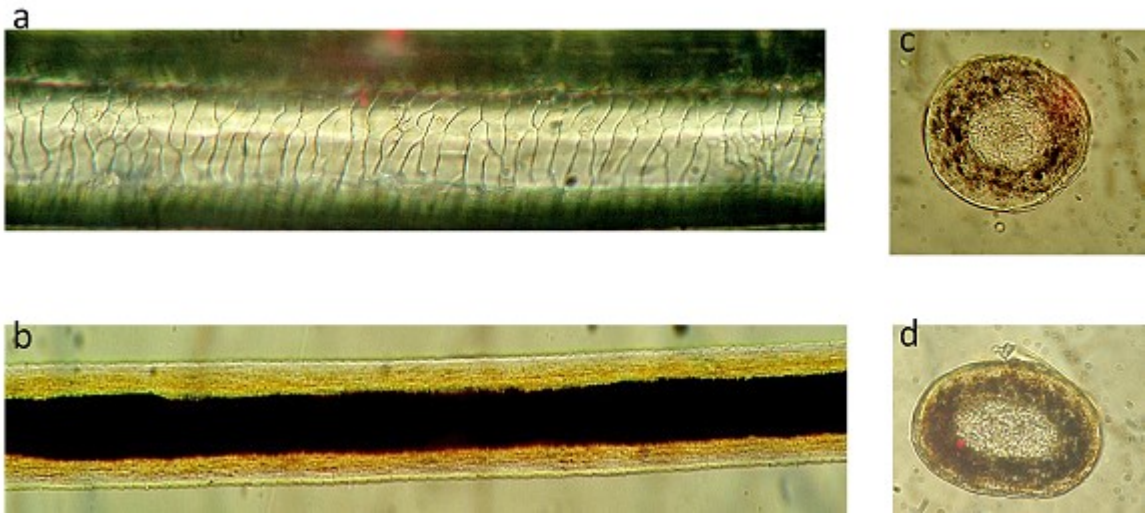


Fig. 4.8 Microphotographs (all 400X) of guard hair of cow/ox. a, predominant scale pattern; b, predominant whole mount; c and d, cross-section

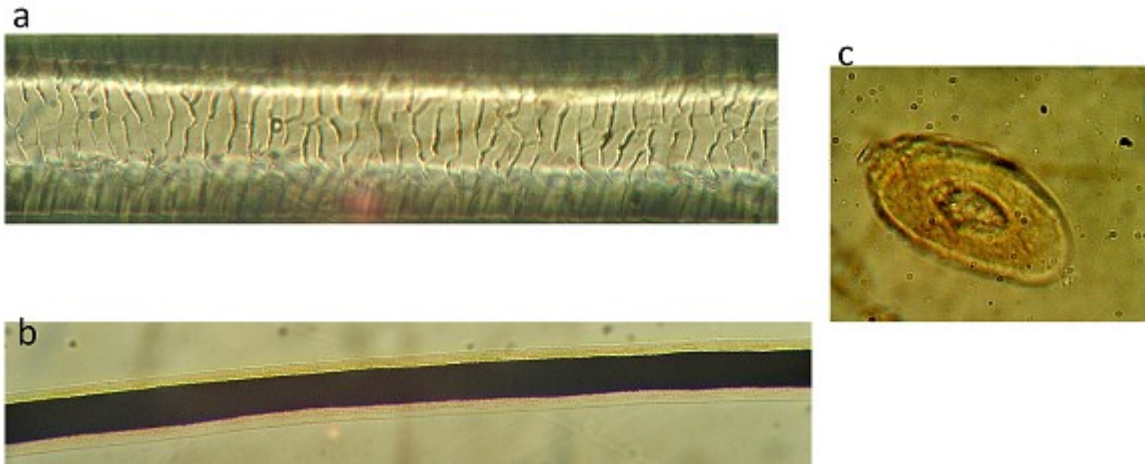


Fig. 4.9 Microphotographs (all 400X) of guard hair of horse. a, predominant scale pattern; b, predominant whole mount; c, cross-section

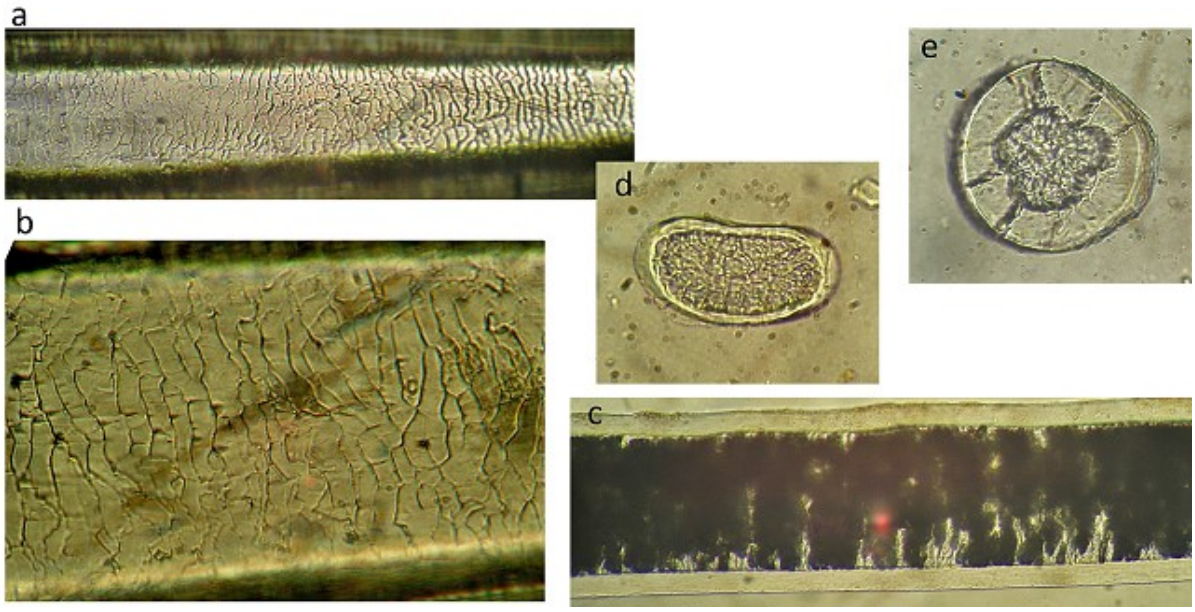


Fig. 4.10 Microphotographs (all 400X except a) of guard hair of domestic goat. a and b, predominant scale pattern; c, predominant whole mount; d and e, cross-section

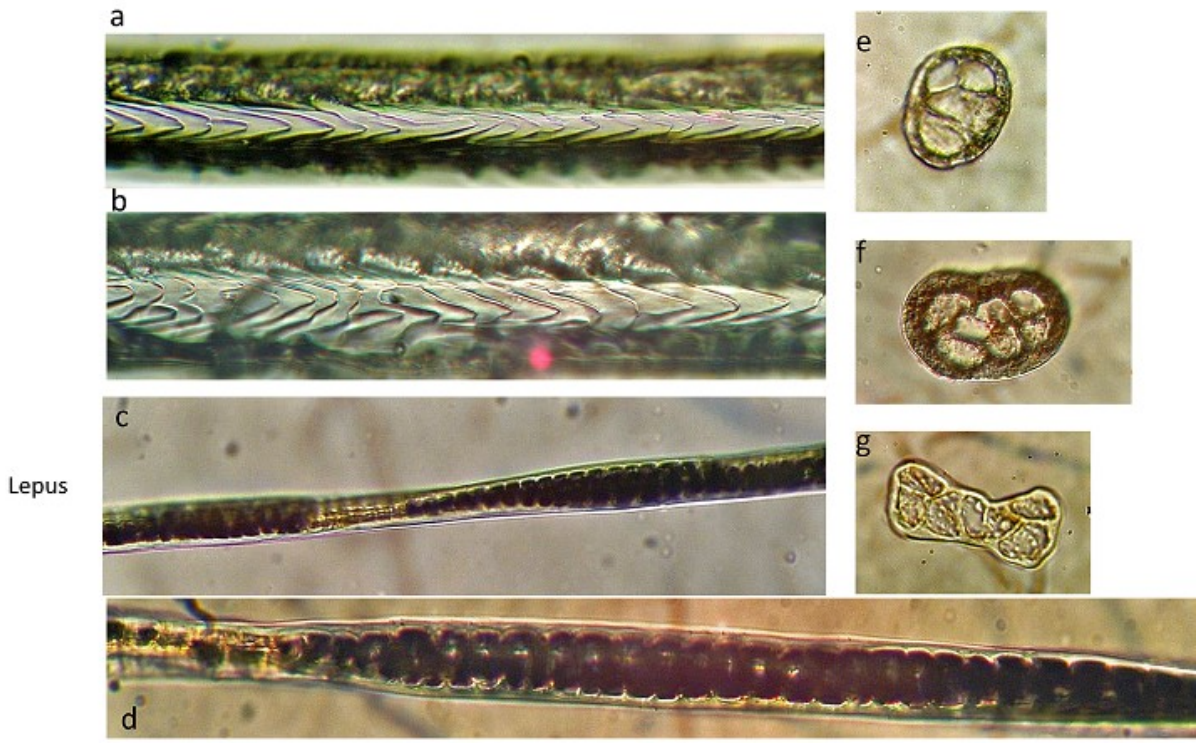


Fig. 4.11 Microphotographs (all 400X except a 200X) of guard hair of woolly hare. a and b, predominant scale pattern; c, predominant whole mount; d and e, cross-section

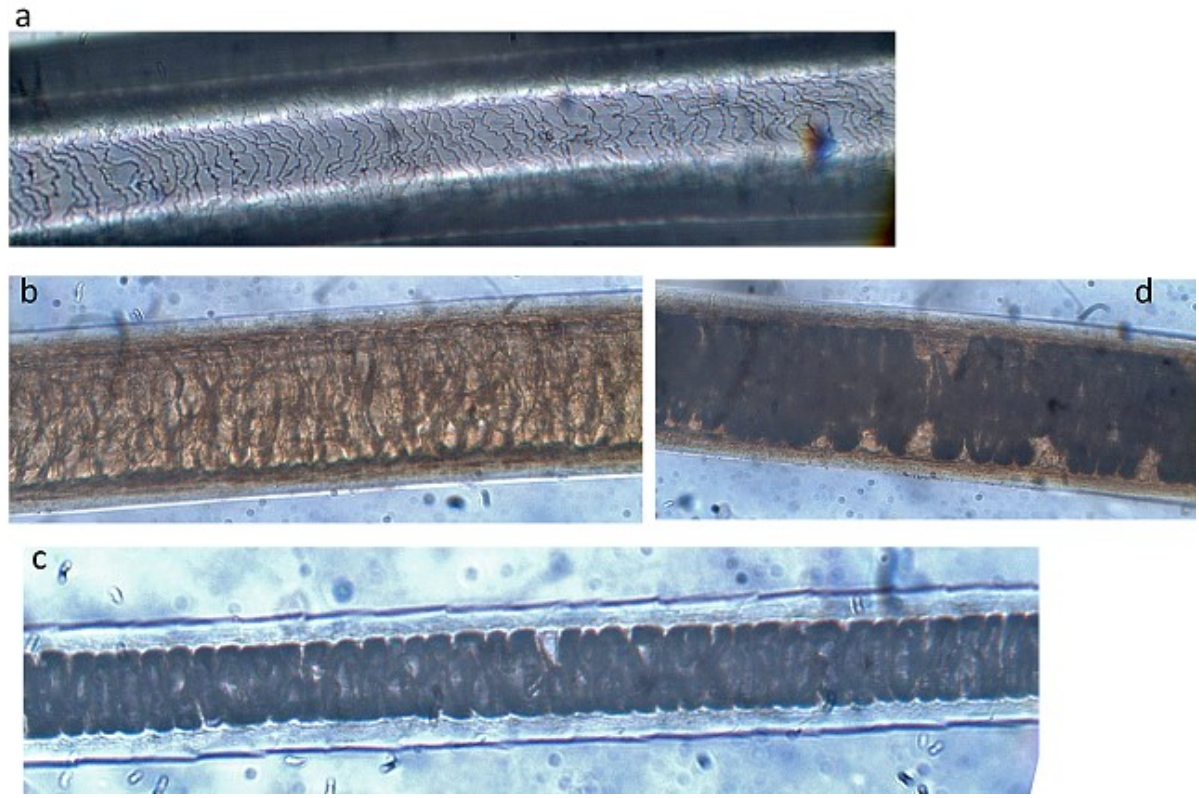


Fig. 4.12 Microphotographs (all 400X) of guard hair of mountain weasel. a, predominant scale pattern; b and d, predominant whole mount; c, whole mount at distal end

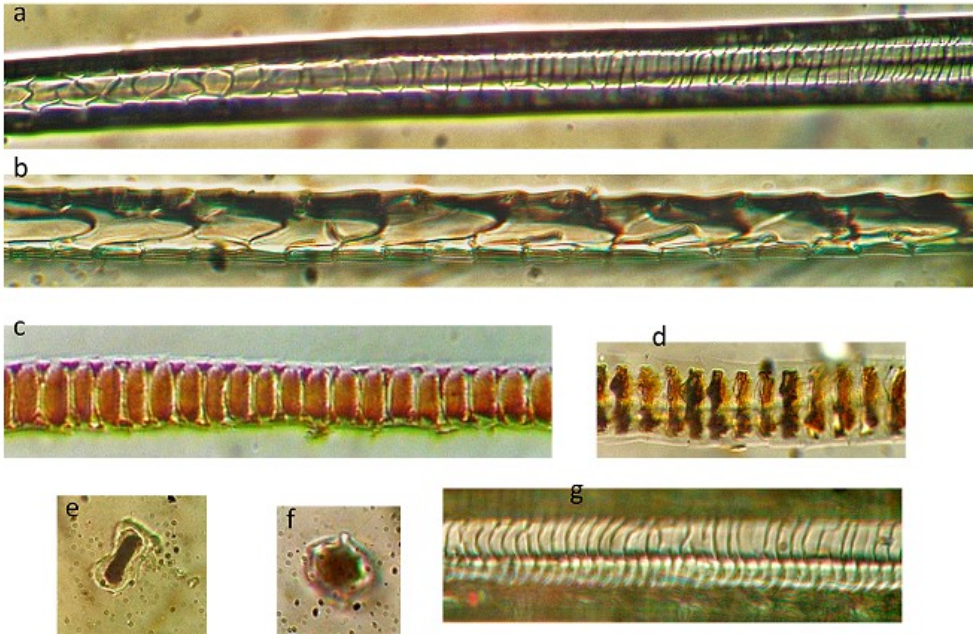


Fig. 4.13 Microphotographs (a, b, e, f and g, 400X; c and d, 200X) of guard hair of shrew. a, transitional scale pattern; b, scale pattern at proximal; c and d, predominant whole mount; e and f, cross-sections; g scale pattern at distal

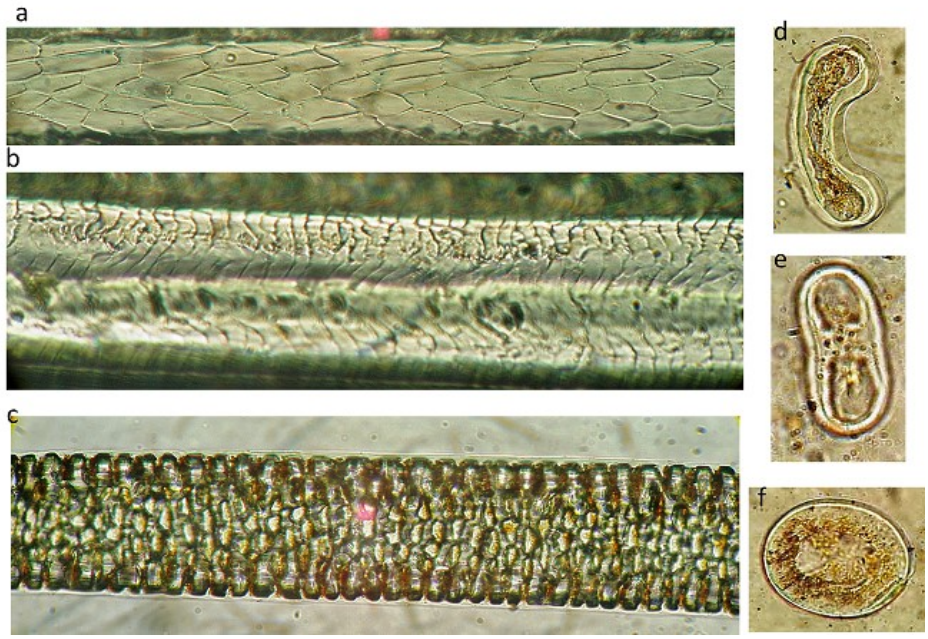


Fig. 4.14 Microphotographs (all 400X except a 200X) of guard hair of rat. a, predominant scale pattern at proximal; b, scale pattern at distal; c, predominant whole mount; d, e and f, cross-sections

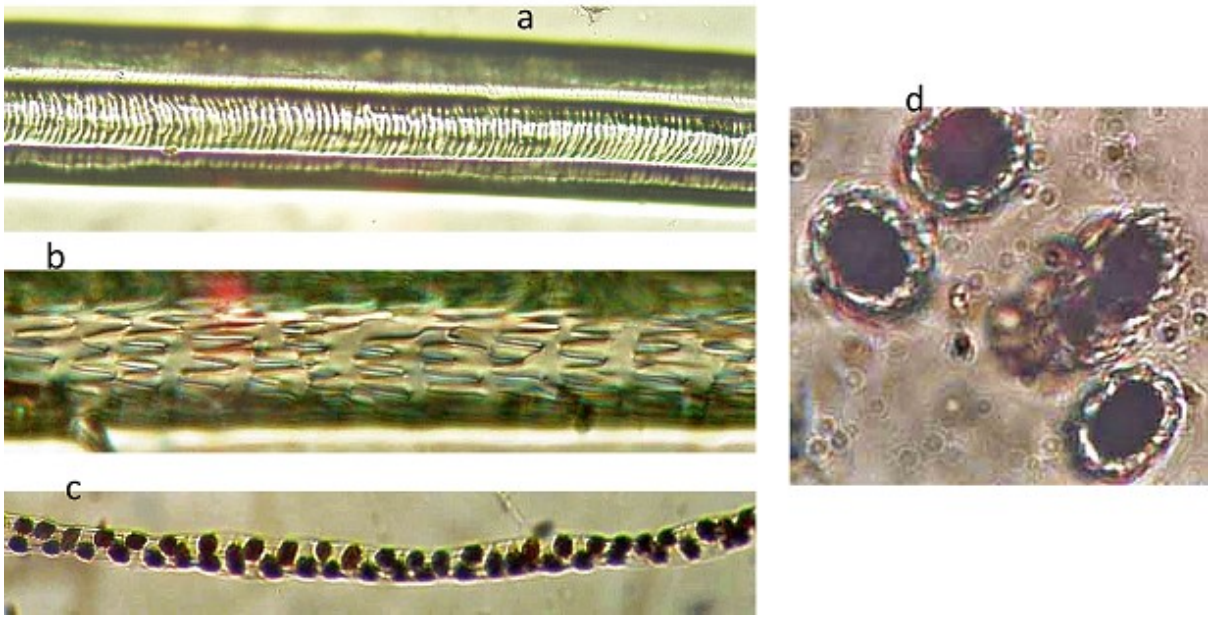


Fig. 4.15 Microphotographs (b and d, 400X; a and c, 200X) of guard hair of pika. a, scale pattern at distal; b, predominant scale pattern at proximal; c, predominant whole mount; d, cross-sections

4.2.3 Key to Prey Species of Snow Leopard in Nepalese Himalaya

A. Overall thick (30 µ to 335 µ) and long hair (3 cm to 30 cm) with wide or medium medulla (large prey species key 1–6)

B. Overall thin (10 µ to 110 µ) and short hair (0.8–3.6 cm) with wide medulla (MI = 0.763–0.88 µ) (small prey species key 7–8)

1 Medulla predominantly fragmented, thin (MI = 0.119) and heavily pigmented, thick hair (70–110 µ). ……**Yak** (Fig. 4.6)

2 Medulla continuous (3 to 7)

3 Medulla lattice and wide (MI = 0.789–0.943 µ) ……..……... go to 3.1 and 3.2

3.1 Scale pattern is broad petal and non-porous cross-section …. **Blue sheep** (Fig. 4.2)

3.2 Scale pattern is flattened irregular mosaic and porous cross-section ……. **Musk deer** (Fig. 4.3)

4 Medulla simple and wide (MI = 0.717–0.788 μ)

4.1 Scale pattern is regular wave, scale and medulla margin are straight or very slightly scalloped, distant scale, cross-section circular and porous ……..... **Himalayan tahr** (Fig. 4.1)

4.2 Scale pattern is irregular wave, scale margin rippled and crenate, medulla margin fringed, close scale, cross-section circular or oval ……. **Goat** (Fig. 4.10)

4.3 Scale pattern diamond pattern, scale margin smooth and dentate, medulla margin regular fissure or fringed, distant scale, cross-section circular or oval …….**Red fox** (Fig. 4.4)

5 Medulla simple and medium width (MI = 0.406–0.531 μ)

5.1 Scale pattern is irregular wave, scale margin rippled or scalloped, medulla margin slightly scalloped, distant scale, cross-section circular …….**Snow leopard** (Fig. 4.5)

5.2 scale pattern regular or irregular, scale margin smooth or slightly crenate, medulla margin straight, scale distant, cross-section circular and oval ……**Cow** (Fig. 4.8)

5.2 Scale pattern regular or irregular, scale margin crenate, medulla margin straight, scale close, cross-section oval ……...**Horse** (Fig 4.9)

6 Medulla simple or uniserial ladder, medium width (0.319), scale pattern is regular wave, scale margin smooth or slightly crenate, distant scale, cross-section circular…. **Dog** (Fig. 4.7)

7 Medulla non-simple and wide

7.1 Scale margin smooth and dentate, distant scale, scale pattern regular wave or chevron, medulla margin scalloped margin, cross-section oval or biconcave ……...……**Woolly hare** (Fig. 4.11)

7.3 Scale margin dentate and slightly crenate, close scale, scale pattern diamond petal and irregular wave, medulla lattice, elongated fringed, scalloped margin, cross-section circular or biconcave …….. **Rat** (Fig. 4.14)

7.5 Scale margin rippled, close scale, scale pattern irregular wave, medulla unbroken vacuolated, irregular uniserial ladder, scalloped margin, cross-section circular or oval ……..……..**Mountain weasel** (Fig. 4.12)

8. Medulla non-simple, discontinuous and wide

8.1 Scale margin smooth and dentate, distant scale, scale pattern diamond petal and transitional, medulla elongated uniserial ladder, cross-section circular or biconcave ....……..……**Shrew** (Fig. 4.13)

8.2 Scale margin smooth and dentate, close scale, scale pattern narrow diamond petal and irregular wave, medulla irregular stacked, double bands or ladders, cross-section circular ……..… **Pika** (Fig. 4.15)

4.3 Conclusion

Prey hairs in predator's faeces can serve as a reliable and an important tool to study food habits of carnivores. This study shows hair microphotographs with a reference key including macroscopic note and microscopic examination of cuticular scales and medullary type, thickness of cortex and medulla, medullary index etc., as a suitable diagnostic tool for identifying species of the prey. Compared to direct comparison and using the dichotomous key, reference to photographs also proved more convenient and easier procedure for the routine identification of hair.

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Paper VI

Diet and prey selection by snow leopards in the Nepalese Himalayas

Bikram Shrestha, Joxerra Aihartza and Pavel Kindlmann

(see attached published paper)

Paper VII

Snow leopard-human conflict and effectiveness of mitigation measures

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ABSTRACT

Understanding the dynamics of snow leopard-human conflicts and the perceptions of local people of the threats posed by snow leopards is important for gaining local support for mitigating the effects of the conflicts. In this chapter, we assess the knowledge and perception of local people of livestock losses due to snow leopards in the central and north-eastern Himalayas in Nepal. In nine settlements in three protected areas (Annapurna Conservation Area – ACA, Manaslu Conservation Area – MCA and Sagarmatha National Park – SNP) we studied, how the perception of local people of snow leopard depredation has changed over time and its correlation with livestock losses. We carried out questionnaire-based interviews of 1015 households from 2004 to 2016, which included 26.45% to 100% of all households in the settlements. Herding of yak/nak (nak is female yak), sheep/goats and cattle (cows, oxen and horses) were found to be the main sources of livelihood for all households in the villages. Herders reported losses of livestock mainly due to attacks by snow leopard and two other carnivores (wolf and lynx). Most (1.5% to 14.3%) losses were attributed to snow leopard, while the other predators accounted for a meagre 0.16% to 5.3%. Predator-induced loss was substantial for the local families and reached $349 per household per year. However, livestock mortality due to other causes (disease or natural disasters) was higher than that attributed to predators. We also evaluated the effectiveness of existing mitigating programmes, described community-based local mitigation measures and assessed the subsequent reduction in the level of conflict. This revealed that the number of conflicts was lower than in 1990 and 50% of the respondents had changed their mind about snow leopard conservation. Surprisingly many respondents (15%) were against the conservation of snow leopards and even considered retaliatory killing of this predator as the best solution. Of the five snow leopard-human conflict mitigation measures, compensation from a community-based livestock insurance scheme and the improving of animal husbandry were the most popular in all the regions studied. Altogether, 15 human-induced threats to the future survival of snow leopards and its wild prey were identified in two protected areas. We conclude that there is still a major threat to the long-term survival of snow leopards and its natural prey in the areas studied. Mitigation measures

identified during discussions with local people should be applied to create a win-win situation for both local people and the long-term survival of snow leopards.

8.1 Introduction

Snow leopard (*Panthera uncia*) inhabits alpine and sub-alpine meadows at altitudes of 2,500–5,500 m in the Himalayas in Nepal. It prefers a steep terrain, well broken by cliffs, ridges, gullies and rocky outcrops (Jackson and Ahlborn 1989; McCarthy and Chapron 2003).

In the Himalayas above 3,000 m, where the dominant vegetation is grass and alpine shrubs the majority of the people there are still dependent on animal husbandry for their livelihood (Miller 1995; Richard et al. 2000; Gurung and McVeigh 2002; McVeigh 2004), especially in areas away from trekking routes. Livestock is important in their day-to-day life and culture and livestock herding is their main economic activity, as they have limited livelihood opportunities due to shortness of the summer season, which limits them to one crop per year (MOAC 2011). However, livestock and snow leopard share the same habitat. Because of this, snow leopards frequently encounter and kill livestock and this can have a substantial effect on the local economy (Oli et al. 1994; Jackson et al. 1996; Bagchi and Mishra 2006; Shrestha 2006; Shrestha et al. 2012; Li et al. 2013; Suryawanshi et al. 2013; Ale et al. 2014; Alexander et al. 2015). Depredation of livestock is a major problem in the Himalayan region and is often reported in the local press (Fig. 8.1). The loss of livestock due to attacks by snow leopards has resulted in them being viewed as vermin that need to be eradicated (Din et al. 2017). Understanding the predation pressure on livestock and the existing ecological and social issues associated with human-snow leopard conflicts is important for developing effective means of managing and conserving large carnivores in habitats used for livestock grazing (Bagchi and Mishra 2006).

Oli et al. (1994) and Jackson et al. (1996) report the incidence of livestock losses due to attacks by snow leopards and its effect on the human perception of this predator in Upper Manang in the Annapurna Conservation area (ACA) in 1990 and 1992. Shrestha et al. (2012) report the killing of livestock by snow leopards at Phortse in the Sagarmatha National Park (SNP) during 2005–2006. Similarly, Ale et al. (2014) report the killing of livestock in Lower Mustang, ACA, during 2010 and 2011. The incidence of killing, however, is highly site dependent, differs from site to site, between years, with changes in the pattern of livestock

herding, and the density and behaviour of snow leopards (Jackson 2010; Suryawanshi et al. 2013).

It is therefore urgent to study, how the conflict between local people and snow leopards is changing over time, and determine the major factor responsible for heightening this problem. Our study aims to compare the current level of predation and the conflict between snow leopards and people with that recorded two decades ago in Upper Manang and Sagarmatha National Park (Oli et al. 1994; Jackson et al. 1996). Moreover, we also assess the pattern in the killing of livestock in other snow leopard habitats.

Because of the human-wildlife conflict, there have been some attempts to tackle the problem in both ACA and SNP, however there are no assessments of whether they have been effective or whether the attitude of locals towards snow leopard has changed. In view of the above, this study also aims to evaluate the effectiveness of the attempts to intervene in the conflict and identify local community-based measures to mitigate the conflict and ensure the long-term survival of snow leopards in SNP and ACA.

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Snow leopard kills 27 Himalayan goats

- RSS, Mustang

Jan 5, 2016-

At least 27 Himalayan goats died after a herd of snow leopards attacked the goats at Tetang village of Chhusang VDC-5 in upper Mustang.

Chief of LhoManthang Office of Annapurna Conservation Area Project (ACAP) Santosh Sherchan said that the snow leopards killed 27 Himalayan goats and injured two others by entering into the barn of farmer Ram Bahadur Gurung on Saturday night.

After the death of a large number of goats, farmer Gurung has suffered a huge loss.

As the campaign to conserve the snow leopard came into effect some four years ago, number of the beast has increased in the upper Mustang.

Sherchan said that the snow leopards enter into the villages for lack of preys in the jungle along with the rise in their number.

Earlier in September, the snow leopards had entered into the village and killed over 100 Himalayan goats of farmer resident Pemachheten Gurung at Marang village in Charang VDC.

ACAP Chief Sherchan said that the farmers would not have to bear losses from the snow leopard after the solar lights installation at animal sheds. "We are running awareness raising programmes targetting the locals," he added. RSS

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Fig. 8.1 A local newspaper report of snow leopards killing 27 Himalayan goats. (http://kathmandupost.ekantipur.com/news/2016-01-05/snow-leopard-kills-27-himalayan-goats.html)

8.2 Study Area

The study areas are located in the following five snow leopard habitats in Nepal, the first three of which are in the Annapurna Conservation Area (ACA):

(i) Lower Mustang (LM),
(ii) Upper Mustang (UMS),
(iii) Upper Manang (UM),
(iv) Tsum Valley (Chhekampar VDC), Manaslu Conservation Area (MCA),
(v) Sagarmatha National Park (SNP).

For details of these areas see Chapter 9.

8.3 Methodology

Questionnaire survey and threat assessment were used to study the level of existing snow leopard-human conflict in the study area.

8.3.1 Questionnaire and Survey

A well prepared questionnaires were used to obtain information on household size, livestock number and type, number killed by snow leopards and the estimated economic loss due to predation over the last 12 months.

These data were categorized and means and percentages calculated and illustrated graphically and in tables using MS Excel 2016.

For determining the attitude of local people to snow leopards, a multiple-choice questionnaire with answers constrained to either disagree, neutral, agree or strongly agree, or yes, no or why. Below are the five most important measures aimed at mitigating the snow leopard-human conflict,

(i) improved husbandry (guarding of herds, corralling animals in predator proof enclosures at night, use of dogs, electric fencing, keeping a close watch and warding of predators),

(ii) providing compensation through a community-initiated livestock insurance scheme,

(iii) wildlife-related measures (prey conservation, complete extermination of snow leopards, killing only those that attack livestock),

(iv) livestock-related measures (avoid grazing in areas in which there is a high risk of predation, reduce the number of livestock),

(v) incentive-based programs (saving and credit schemes, conservation advocacy, wildlife-based tourism, veterinary service for livestock, devices for deterring predators such as fox-lights and sound alarms; for each option, a scale from 1 to 3 was assigned:1 – least important, 2 – moderately important, 3 – most important. The final value was the mean of these values.

Household survey was conducted in order to determine the number and type of livestock and the cause of their death in nine local areas from 2004 to 2016 (Table 8.1). In UMS, all of the 329 households in three settlements (Lo-Manthang, Chhoser and Charang) were surveyed, in LM, all of the 44 households in two settlements (Jhong and Lupra) and in UM, 50 out of 189 households in two settlements (Khangsar and ProperManang). In MCA, all 257 households in Chekampur VDC in Tsum valley were surveyed. In SNP, all 75 households in the settlements there were questioned. We compared our results with data obtained by previous researchers and published results in order to determine the extent to which the numbers have changed.

8.3.2 Threat Assessment

We identified the human-related factors that negatively affect snow leopard and its wild prey in order to determine the threat to conserving snow leopards in three regions. This was based on field observations and focus groups discussions carried out from 2004 to 2015 in SNP and 2010–2016 in LM and UM (Table 8.2). The threats were ranked based on the area (proportion of the habitat(s) at a site that is threatened), intensity (severity of the threat) and urgency (immediacy of the threat), following Margoluis and Salafsky (2001). We identified 12 threats at each of three sites LM, UM and SNP. For a particular threat, a score was assigned ranging from the lowest (indicated by 1) to most severe (indicated by a number equal to the total number of threats in the region). Thus, for example, in LM, there were 12 threats and scores ranging from 1–12 were assigned, with 1 indicating the "lowest" and 12 "most severe". Thus, the ranking of the threat (sum of scores for area, intensity and urgency) in LM was between 3 (= 3×1) and 36 (= 3×12). When calculating the average threat level, we considered scores of from 1 to 12 to indicate low threat, 13 to 24 medium threat and 25 to 36 severe threat. Likewise, in UM and SNP the scores for the different categories of threat are similar to that recorded for LM because12 threats are recorded for each of these sites.

Percentage reduction in the level of threat was estimated based on a discussion of the degree to which a particular threat had been reduced by conservation activities at the end of the assessment period as explained in Table 8.3. What does 100% reduction in the level of a threat mean? For example, in the case of Lower Mustang, no snow leopards were killed by local people at the end of the assessment period. For reducing the adverse effect of livestock on the abundance of the natural prey of snow leopards it is important to implement a policy of sustainable grazing by livestock by regulating the use of pasture land.

The raw score is defined as the total rank times the percentage reduction in the threat. For example, convert 50% to 0.5 before multiplying it by 29 to get the total raw score of 14.5 for retaliatory killing of snow leopards by local people in the case of Lower Mustang. Then the total raw score is the sum of raw scores for each threat. While this raw score is not of any specific significance, it is critical for the calculation of the reduction in the level of a threat (Margoluis and Salafsky 2001, page 31).

Finally, the threat reduction assessment index (TRA) was calculated by dividing the total raw score by the total rankings of all the threats and multiplying by 100: (TRA = $\sum$ total raw scores / $\sum$ total rankings $\times$ 100) (Margoluis and Salafsky 2001). Thus, the TRA in principle

describes the efficiency of the measures used to reduce the level of threat in a region. It is an indirect measurement of the effect of conservation. TRA is a summary indicator of the degree to which a project has succeeded in reducing the threats to conservation at a particular site. If TRA is close to 1 (100 % threat reduction), then the measures used to reduce the threat were very effective, but if TRA is close to 0 they were not effective.

8.4 Results

8.4.1 Livestock Mortality

Based on interviews with herders in nine settlements in three protected areas: ACA, MCA and SNP (Table 8.1), livestock losses due to snow leopards ranged from 1.5% to 14.3% of total stock per annum. The highest livestock mortality due to snow leopards was recorded in Proper Manang, followed by Khangsar, Lupra, Phortse, Chhoser and the lowest in Lo-Manthang, Jhong, Chhekampar VDC and Charang. There was an increase in the trend in percentage loss from 2.9–4.2% in 1990–1992 to 10.3% per annum in 2015 in Khangsar (UM) and from 1.6% in 1990 to 14.3% per annum in Proper Manang. In Phortse (SNP), the trend increased from 1.3% in 2004 to 4.8% in 2007, and then decreased to 2.5% in 2015. From 2011 to 2016, the trend decreased slightly in Lupra and increased slightly in Jhong.

The loss due to other predators, such as wolf, lynx, red fox or golden jackal, ranged from 0.16 to 5.3% per annum in the nine settlements. The livestock losses from all predators ranged from 0.2 to 3.5 per household per annum. On average, the monetary loss due to predators amounted to NRs. 34880 (USD 349) per household per year in Upper Manang. The livestock mortality due to causes other than predators (disease, starvation, fighting, falling, avalanches etc.) was highest in UM (range: 24.9–33.1%), followed by UMS, LM, MCA and SNP (range: 1.5–8.8%).

The loss of livestock due to snow leopards, other predators and other causes were more or less equal in UMS. In LM and SNP, people did not think the other predators significantly affected their losses of livestock, while mortality due to other causes was reported to be greater than that due to snow leopards. In UM, the people similarly did not think the other predators significantly affected their losses of livestock over the period 1990 to 1992, but reported higher losses in 2015, mainly due to causes other than predation.

8.4.2 Livestock Herding

In UMS, goats/sheep are the major livestock with an average of 30.6 animals/household (HH) and a range of 26.3 to 35.5 animals in 2012. The next in importance was cattle (3.7 animals/HH; range: 2.9 to 5.1), yak/nak (1.7 animals/HH; range: 0.03 to 5.6) and horses/mules (1.7 animals/HH; range: 1.4 to 2.1) (Table 8.1).

In Lupra in LM, goats/sheep are the major livestock, which increased from 27.7/HH in 2011 to 45.8/HH in 2016. Cattle also increased from 3.1/HH in 2011 to 5.2/HH in 2016. Similarly, in Jhong in LM, goats/sheep are the major livestock but decreased slightly from 24.8/HH in 2011 to 22.8/HH in 2016. Cattle increased from 3.1/HH in 2011 to 7/HH in 2016. In both Lupra and Jhong, yak/nak and horses/mules decreased from 1.1/HH in 2011 to 0/HH in 2016 and from 1.2/HH in 2011 to 0.6/HH in 2016, respectively.

In Khangsar in UM, yak/nak are the major livestock, which increased from 3.48/HH in 1992 to 16.2/HH in 2015. Goats/sheep were the major livestock in 1990–1992 but were not present in 2015. In Proper Manang in UM, yak/nak were the major livestock and increased from 5.2/HH in 1990 to 10.5/HH in 2015. Goats/sheep decreased from 20.1/HH in 1990 to 4/HH in 2015. In both Khangsar and Proper Manang, the numbers of cattle and horses/mules remained constant from 1990 to 2015.

In Chekampar VDC in MCA, cattle are the major livestock (4.7/HH) followed by yak/nak (3.9/HH), horses/mules (1.3/HH) and goats/sheep (0.1/HH).

In Phortse in SNP, yak/nak are the major livestock and increased slightly from 4.6/HH in 2004 to 6.3/HH in 2015. Similarly, cattle increased slightly from 1.7/HH in 2004 to 2.1/HH in 2015. Horses/mules were fewer in number and ranged from 0.01/HH in 2004 to 0.04/HH in 2015. Goats/sheep were not kept in SNP. People in Phortse did not own any horse in 2007 and 2015.

Table 8.1 Livestock status and mortality due to predation and other causes recorded in five different regions in Nepal (HH – household, SL– snow leopard, OP – other predators, OC – other causes, NA – not available)

			Type of livestock					Livestock mortality per year due to						Loss to predators per year
Village	Year	HH	Sheep/Goats	Cattle	Yak/Nak	Horses/Mules	Total	SL	%	OP	%	OC	%	per HH
Upper Mustang														
Lo-Manthang	2012	87	2,498	249	491	186	3,424	80	2.3	55	1.6	53	1.5	1.6
Chhoser	2012	130	4,610	389	4	183	5,186	137	2.6	83	1.6	102	2.0	1.7

Tsarang	2012	112	2,942	571	78	191	3,782	55	1.5	33	0.9	116	3.1	0.8
Total		329	10,050	1,209	573	560	12,392	272	2.2	171	1.4	271	2.2	1.3
Lower Mustang														
Lupra	2011	14	388	43	15	17	463	29	6.3	0	0	28	6.0	2.1
Lupra	2016	12	550	62	0	7	619	30	4.8	0	0	31	5.0	2.5
Jhong	2011	30	744	92	37	12	885	17	1.9	0	0	55	6.2	0.6
Jhong	2016	30	685	210	0	7	902	21	2.3	0	0	32	3.5	0.7
Upper Manang														
Khangsar	1990	29	388	145	169	33	735	21	2.9	0	0	NA	NA	0.7
Khangsar	1992	69	915	285	240	60	1,500	126*	4.2	0	0	74*	4.9	1.4
Khangsar	2015	22	0	105	357	31	493	51	10.3	26	5.3	123	24.9	3.5
Proper Manang	1990a	52	1,047	50	272	89	1,458	23	1.6	0	0	NA	NA	0.4
Proper Manang	2015	28	111	49	294	42	496	71	14.3	23	4.6	164	33.1	3.4
MnasluCA (MCA)														
Chekampar VDC	2012	257	26	1,211	1,004	331	2,572	45	1.7	4	0.2	227	8.8	0.20
SNP														
Phortse	2004c	72	0	123	331	2	456	7	1.3	0	0	27	6	0.1
	2006d	73	0	142	334	1	477	16	3.4	0	0	29	6.1	0.22
	2007	73	0	152	364	1	517	25	4.8	0	0	26	5	0.3
	2015	75	0	159	470	3	632	16	2.5	0	0	28	4.4	0.2

Lower case letter attached to years: a – Oli 1991 and Oli et al. 1994, b – Jackson et al. 1996, c – Shrestha 2004, d – Shrestha et al. 2012, without sign – this study, *– livestock mortality over a period of 24 months

Herd sizes of households ranged from 5 to 250 goats/sheep in ACA (LM, UMS and UM), from 1 to 40 in SNP and from 1 to 10 in Jhong and Lubra (LM), and from 5 to 100 yak/nak in settlements in UM. In LM and UM, all the goats/sheep from one village were grouped into 2 or 3 herds ranging in size from 150 to 300 individuals. Similarly, in UM, two to four herders grouped their yak/nak into one herd ranging in size from 150 to 200 individuals, while in LM and SNP each herder herded their own yak/nak.

Livestock herding pattern was similar in all areas in consisting of two to four seasonal rotations between different pasturelands. Some herds were kept at high altitudes in summer and low altitudes, usually close to the village, in winter. Some herds were moved between three or four pasturelands during the course of a year. Autumn and spring pasturelands were located between those used in summer and winter. In Namche (SNP), the people do not have a traditional rotational grazing system and let their animals roam freely all year round. In winter, most of the livestock were brought down to lower altitudes or kept in sheds next to their houses at night, where they were provided with hay or wheat straw. During the course of discussions with local herders, those that rear livestock reported that there was a scarcity of green grass over the last few years due to low rain fall and little snow. Due to this and global warming,

cattle were not moved to low altitudes in winter. Therefore, some herders in Thini (LM) and Phortse (SNP), keep their yak/nak in high pastures even in winter.

All herds of goats/sheep were moved by one or two herders from place to place, keeping them in closed corrals at night. Some herders had a Tibetan dog to guard the herd. Herds of female yak (nak) with calves were left free to roam in pasturelands during the day and kept overnight in nearby fallow lands next to a Goth (a temporary shed where herders cook and stay overnight) or in poorly built open corrals. At lower altitudes, the livestock was kept in sheds often next to the villagers' houses at night. During the night, the calves were kept inside either in the open corrals or depending on their availability in predator-proof corrals. Yak (adult male) were allowed to roam free in pasture lands throughout the year. During summer, horses were free to roam in pasture lands and used for transporting hay or tourists.

8.4.3 Local People's Knowledge and Perception of Snow Leopards

Almost all the respondents (70%) in all the areas studied believed that snow leopards do not attack humans but definitely attack livestock. More than 50% of them agreed that snow leopards are beautiful animals, but only 25–35% thought they would attract tourists. The majority of the respondents (85%) were aware that they are protected and that it is illegal to kill them. About 40–60% believed snow leopards are protected for religious reasons (Buddhist faith).

In terms of whether snow leopard should be conserved or not, many of the respondents were indifferent (38−55%), followed by those who agreed (20–36%), strongly agreed (10−16%) and disagreed (8−15%) (Fig. 8.2). Opinions of literate and illiterate people on whether snow leopards should be protected in UM differed significantly (Chi square = 28.8, df = 3, P = 0.00).

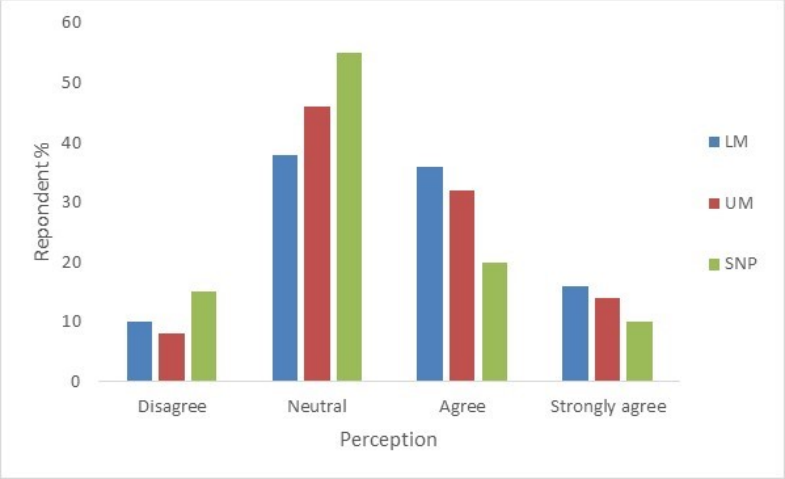

Fig. 8.2 Local people's opinion on whether snow leopards should be protected

8.4.4 Ways of mitigating Snow Leopard-Human Conflict Suggested by Local People

The people's perception of how to mitigate human-snow leopard conflicts, were recorded in four of the regions studied. They were asked to choose one of the following five options:

(i) Improving husbandry (close guarding of herds, corralling animals in predator-proof enclosures at night, use of dogs, electric fencing, creating a disturbance and chasing leopards away).

(ii) Providing compensation through community-based livestock insurance schemes.

(iii) Wildlife-related measures (prey conservation, complete extermination of snow leopards, killing only those that attack livestock).

(iv) Livestock-related measures (avoid grazing in areas of high predation risk, reduce the number of livestock).

(v) Incentive programs (snow leopard saving and credit program, handicrafts or eco-tourism, a veterinary service, use of lights that deter predators, such as fox-lights etc.).

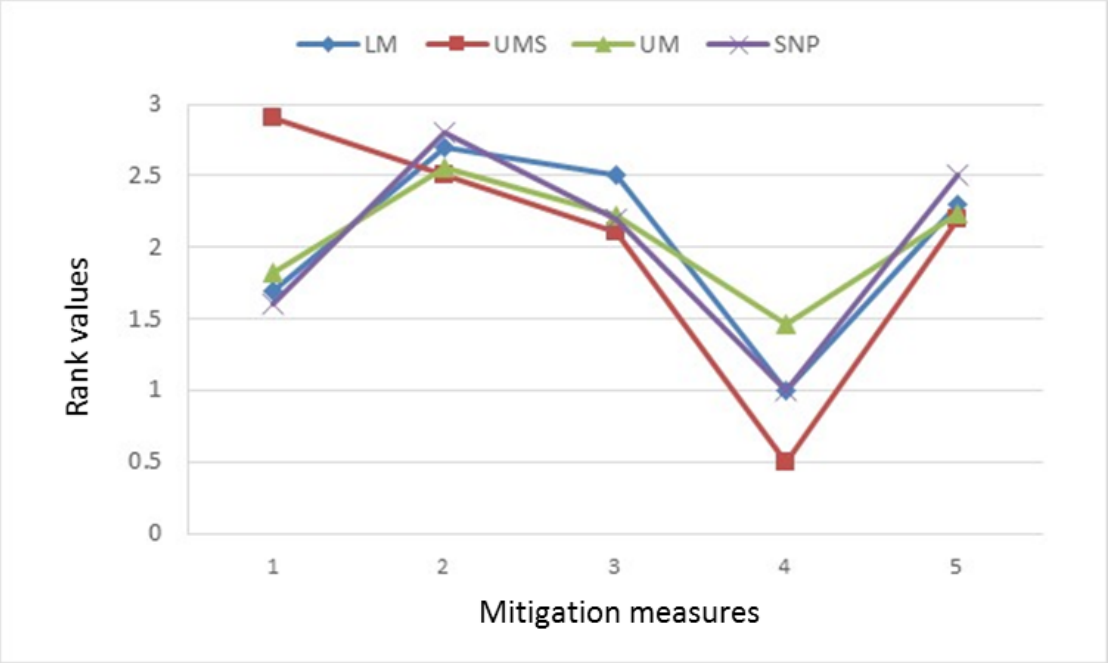

Fig. 8.3 People's perception of how to mitigate human-snow leopard conflicts recorded in four regions. The options were: (i) improving husbandry (ii) providing compensation through community-based livestock insurance schemes, (iii) wildlife-related measures, (iv) livestock-related measures, (v) incentive programs

All respondents agreed it was necessary to control the loss of livestock due to predation by snow leopards. Of the five mitigation measures option (ii) was the most voted for in all regions except UMS, where most people voted for option (i). Wildlife-related measures (option (iii)) and incentive programs (option (v)) receive more or less equal votes and second-best choice of five options in the three regions (LM, UM and SNP) while these options stand as third-best choice in UMS. In three of the regions studied (LM, UM and SNP), improving husbandry (option (i)) was voted the third-best way of mitigating conflict. Livestock-related measures (option (iv)) was the most dislike option among five options in all four regions (Fig. 8.3).

8.4.4 Mitigation Measures Adopted

8.4.4.1 Livestock insurance scheme

In UM, 45 of 50 the respondents (90%) were aware and only 10% were unaware of the livestock insurance scheme (LIS) provided by the Conservation Area Management Committee (CAMC) in collaboration with Annapurna Conservation Area Project (ACAP). Compensation was provided only for the loss of livestock killed by snow leopards. Thus, of the respondents, only

23 had applied for compensation and only 17 of them had received compensation in 2014. However, in 2015, there were no claims for compensation. In UM, each village development Committee (VDC) received NRs 125,000 to provide compensation in the first year and additional NRs 50,000 in successive years. Local herders are required to insured their livestock in order to claim for compensation. The insurance premiums are NRs. 100, 50, 25, 10 and 10, respectively for horse/mule, yak/nak (1−3 years old), zhopa (hybrid between yak and cattle), goats/sheep and cows/oxen. If insured livestock are killed by snow leopards, the herder receives 20% of the cost of a goat/sheep, yak-cow calf (1−3 years old) and zhopa, and 15% of the cost of a horse/mule. This scheme was not effective, as the compensation was much lower than the loss and the process of claiming was too cumbersome, as it was necessary to provide evidence of a kill for verification, which is sometimes very difficult.

8.4.4.2 Partial compensation schemes

Similarly, most herders were not interested in the partial compensation scheme provided by Lower Mustang's conservation office. They complained about the procedure, the time it took to process their claim and that the value of the compensation was meagre compared to that of the lost livestock. In 2015, only 9 local herders in LM were partially compensated by a payment of NRs 60,400 for 23 animals (16 goats, 3 sheep, 2 yaks/calves, 2 horses) killed by snow leopards. Only 10% of the total cost of the livestock killed was paid out in compensation in LM, where the usual compensation price for goat/sheep, yak-calf and horse are 2000, 8000 and 10,000 rupees, respectively. This compares unfavourably with the total annual budget allocated for partial compensation, which was only NRs 200,000 per year in LM. In LM, the partial compensation scheme is used only when LIS is not available.

In contrast, in SNP, no villagers were aware of the partial compensation scheme under the Government of Nepal. Only one herder had applied for compensation for the loss of 4 yak calves in Gokyo valley in spring 2015. However, his request was still pending at the end of this study (December 2015).

In 2010, a public project called the "saving and credit village program" was established in four villages in SNP in collaboration with the Buffer Zone Management Committee (BZMC) – SNP, which aimed to support snow leopard conservation. Each village received NRS 200,000 as seed money. Each member of the group was asked to save NRS 100 per month. This money was used for standard loans etc. and 5% of the total interest on the money saved was allocated

for funding the partial compensation for losses of livestock due to snow leopards (the rest was distributed among the members).

After the first two years, however, the financing of the project from outside ceased. Since then, the project was solely under the control of the villages, which were inclined towards maximizing their gain (getting most of the interest) rather than providing compensation for domestic animals killed by snow leopards and in this way supporting their conservation. This became especially apparent after 2015, when the money deposited by the members of the group started to exceed the seed money. Thus, only NRs 500 was provided as compensation for each animal killed by snow leopards in 2015, and only one member in Phortse applied for compensation.

The main problem is probably that this program and its supervision is not involved in the institutional national park's framework and as a consequence income-generating schemes that contribute to the conservation of biodiversity are inadequate. Besides the saving and credit programmes run by public fund, the Wildlife Damage Relief Guidelines prepared by the Government of Nepal also provide partial compensation for damage caused by wild animals (GoN 2017). In the later case, villagers should claim or apply the partial compensation to the Nepal Government through national park office.

8.4.4.3 Predator-proof corrals

ACAP has supported local herders in constructing predator-proof corrals. In 2016, ACAP supported the construction of four corrals in selected pasturelands, two in LM, two in UMN, and ~25 in UMS. However, most of them were not repaired and are now non-functional. A well-developed predator-proof corral is a very effective way of reducing the number of livestock killed by snow leopards. As one snow leopard can kill up to100 to150 goats/sheep per night, and as the herders keep moving their livestock to different locations, it makes sense to construct and maintain predator-proof corrals.

8.4.4.4 Distribution of other devices for deterring predators

ACAP has been distributing animal-deterring lights to selected herders in UMS, LM and UM, such as fox-lights and solar lamps ("tukis"), in order to deter snow leopards at night. It is reported, however, that the fox-light is not effective because snow leopards quickly become habituated to the light.

8.4.4.5 Anti-poaching committee

ACAP has established an anti-poaching sub-committee in areas where there is a high incidence of poaching to collect information about suspicious poaching activities and the poachers.

8.4.4.6 Conservation education program

ACAP has produced a book on conservation written in Nepalese called "Prakriti ko sandes" ("Messages from Nature") for school children in classes 6, 7 and 8, which is also widely used for teaching within the ACA region. In addition, on the occasion of wildlife week, ACAP conducts wildlife awareness programmes for school children, such as writing poems and essays, and drawing wildlife and nature. Occasional snow leopard environmental awareness camps and snow leopard street dramas are also staged.

In SNP, an informal school awareness programme was organized in which paintings of snow leopards by classes 1–8 in four schools (Shrestha 2007). In addition, a student campaign called "snow leopard scout" was held on the occasion of world environment day (5 June) at Thame School in 2012, which included painting and a short environmental camp for selected students in grades 6–8 (SLC/NTNC 2014).

8.4.5 Threats to conservation

In total, 15 threats were identified in the three areas studied. The threat assessment (Table 8.2) indicates that the major threats for snow leopards in the areas studied are:

(i) retaliatory killing,
(ii) poaching of animals mainly by porters and labourers who come for short-term employment,
(iii) loss of prey due to competition with livestock and disease,
(iv) disturbance due to the collecting of *Cordyceps* and other plant products,
(v) construction of infrastructure such as roads and hotels/lodges in snow leopard habitats,
(vi) killing of wildlife by free-ranging dogs.

Retaliatory killing, reduction in the abundance of prey due to competition with livestock and the collection of terrestrial plants were identified as severe threats in all three of the regions studied. Collecting *Cordyceps* and low prey abundance due to diseases transmitted by livestock were identified as severe threats in LM and UM. Road construction /maintenance was identified as severe threat in LM, hotels in UM and litter/dung collection and stone mining by

local communities in SNP. The reduction in the threat assessment index was slightly higher in SNP than in the other two regions, in which this index remained approximately the same.

Table 8.2 Threat rankings of Margoluis and Salafsky (2001) recorded in Lower Mustang, Upper Manang and the Sagarmatha National Park, were calculated as outlined in the Material and Methods. Total rank is the sum of all three ranks presented in the columns left of the column "Total rank". Percentage reduction in threat is the degree to which a particular threat was reduced by conservation activities at the end of the assessment period, see Table 8.3. Raw score is the total rank times the percentage reduction in threat expressed as a proportion (see methods). No shading indicates a low level of threat, light grey shading indicates a medium level, dark grey shading with bold font a severe level of threat

Threat	Area	Inten-sity	Urge-ncy	Total rank	Percentage reduction of threat	Raw score
Lower Mustang (LM)						
Retaliatory killing of snow leopards	5	12	12	**29**	50	14.5
Illegal hunting/poaching of snow leopard prey	6	3	6	15	70	10.5
Prey reduction due to competition with livestock	12	11	9	**32**	20	6.4
Prey reduction due to disease transmitted by livestock	10	8	8	**26**	50	13
Effects of road construction /maintenance on snow leopard habitats	8	9	10	**27**	0	0
Hotel/lodge expansion	7	7	5	19	50	9.5
Fuel wood collection by local communities	3	2	1	6	70	4.2
Collection of *Cordyceps* and *Allium* sp (Jimbu)	11	10	11	**32**	0	0
Subsistence collection of terrestrial plants	9	6	7	22	0	0
Unregulated tourism (off-roading/camping/hiking in the wilderness)	4	5	4	13	50	6.5
Illegal fires	1	1	2	4	90	3.6
Killing of wildlife by free-ranging dogs	2	4	3	9	0	0
Total	78	78	78	234		68.2
TRA (Assessment period 2010–2016)						29.1
Upper Manang (UM)						
Retaliatory killing of snow leopards	6	12	12	**30**	40	12
Illegal hunting/poaching of the prey of snow leopards	2	1	1	4	70	2.8
Prey reduction due to competition with livestock	12	10	10	**32**	20	6.4
Prey reduction due to disease transmitted by livestock	10	9	8	**27**	10	2.7
Effects of road construction /maintenance on snow leopard habitats	1	3	2	6	80	4.8
Hotel/lodge expansion	7	8	7	22	70	15.4
Fuel wood collection by local communities	5	7	4	16	50	8
Litter/dung collection by local communities	4	2	3	9	30	2.7
Collection of *Cordyceps* and *Allium* sp (Jimbu)	11	11	11	**33**	0	0
Subsistence collection of terrestrial plants	9	6	9	24	0	0

Unregulated tourism (off-roading/camping/ hiking in the wilderness)	8	5	5	18	20	3.6
Killing of wildlife by free-ranging dogs	3	4	6	13	50	6.5
Total	78	78	78	234		64.9
TRA (Assessment period 2010–2016)						27.7
Sagarmatha National Park (SNP)						
Retaliatory killing of snow leopards	10	12	12	**34**	50	17
Illegal hunting/poaching of the prey of snow leopards	8	3	6	17	70	11.9
Reduction in prey due to competition with livestock	12	11	10	**33**	20	6.6
Reduction in prey due to disease transmitted by livestock	9	4	2	15	20	3
Effects of hydro-electric projects on snow leopard habitats	2	2	3	7	50	3.5
Hotel/lodge expansion	3	7	7	17	50	8.5
Fuel wood collection by local communities	7	5	5	17	50	8.5
Litter/dung collection by local communities	6	10	9	**25**	0	0
Subsistence collection of terrestrial plants	5	9	8	22	0	0
Stone mining by local communities	4	8	11	23	50	11.5
Unregulated tourism (off-roading/camping/ hiking in the wilderness)	11	6	4	21	50	10.5
Killing of wildlife by free-ranging dogs	1	1	1	3	20	0.6
Total	78	78	78	234		81.6
TRA (Assessment period 2004–2015)						34.9

Table 8.3 Explanation of the level of the threats recorded in Lower Mustang, Upper Manang and Sagarmatha National Park

S.N.	Explanation of Threats
1	Threat: retaliatory killing of snow leopards by local people
	100% reduction: stop retaliatory killing of snow leopards
2	Threat: illegal hunting/poaching of snow leopard prey (musk deer/blue sheep/Himalayan tahr) by people for their own consumption and commercial purposes such as sale of musk deer's pod
	100% reduction: eliminate all illegal hunting of animals
3	Threat: reduction in prey due to competition with livestock
	100% reduction: control of livestock grazing by setting up and regulating the management of pastureland by implementing rotational grazing
4	Threat: reduction in prey due to disease transmitted by livestock
	100% reduction: eliminate all contact between wild animals and livestock by establishing a restriction zone
5	Threat: effects of road construction /maintenance on snow leopard habitats – road access increases tourism, which disturbs and restricts the movements of wildlife due to noise and human activity
	100% reduction: document the effect of roads on snow leopards and their prey, identify the connectivity zones and recommend appropriate mitigation measures
6	Threat: hotel/lodge expansion – hotels affect habitat quality, produce solid waste, and the activity of snow leopards is adversely affected by lights and noise
	100% reduction: stop the building of hotels in snow leopard habitats
7	Threat: fuel wood collection by local communities
	100% reduction: collect fuel wood in less sensitive areas and on a sustainable basis

8	Threat: Collection of *Cordyceps* and *Allium* sp (Jimbu) by large numbers of people for commercial purposes has an adverse effect on the habitat and disturbs both snow leopards and their prey
	100% reduction: document the adverse effect of collecting *Cordyceps* on snow leopards and plan to minimize this disturbance.
9	Threat: subsistence collection of terrestrial plants – local people collect terrestrial plants (e.g., mushroom) for their own subsistence, which affects the activity patterns of snow leopards and their prey.
	100% reduction: document the adverse effect of collecting terrestrial plants on snow leopards and plan to minimize this disturbance, forbid the collecting of plants in core areas
10	Threat: unregulated tourism (off-roading/camping/hiking in the wilderness) – increasing trend in eco-tourism is having an adverse effect on the habitat and disturbs snow leopards and their prey.
	100% reduction: mange tourism by restricting camping and hiking in core areas of snow leopard habitat
11	Lighting Illegal fires
	100% reduction: only eliminate all illegal fires, but allow properly controlled fires for defined purposes
12	Threat: killing depredation of wildlife by free-ranging dogs
	100% reduction: remove control all free-ranging dogs from in snow leopard habitats
13	Threat: litter/dung collection by local communities from pastures land has an adverse effective impact on the habitat and affect snow leopard activity
	100% reduction: stop to the collecting of litter/dung from pastures by local communities. from pasture land
14	Threat: Adverse impacts of hydro-electric project's infrastructure on snow leopard habitat
	100 % reduction: Exclusion of hydro-electric project fromin core areas
15	Threat: stone mining by local communities
	100% reduction: controlled of stone mining in snow leopard habitats

8.5 Discussion

8.5.1 Livestock Mortality

The percentage of livestock lost due to snow leopards varied greatly between sites and years. Especially in UM, livestock killed in 2015 was conspicuously greater than from 1990 to 1992 and was higher than at other sites. The variation in livestock mortality due to snow leopards may be due to a combination of factors, such as the abundance of snow leopards and their prey, differences in livestock herding and other reasons. For example, the high level of livestock loses recorded in UM in 2015 may be due to:

(i) increase in snow leopard population from 1990 to 2016 (see Chapter 5),

(ii) increase in the percentage of large livestock made up of calves, such as yak/nak,

(iii) stable blue sheep population during that period (see Chapter 5).

We found that the percentage of livestock losses that can be attributed to snow leopards was in the range of 1.5–14.3%. This is higher than that reported by Alexander et al. (2015) in the Northern region of QNNR in Gansu Province, China (0.3%), but comparable with the 1.3–5.9% reported in Qinghai in China, Baltistan in Pakistan, Ladakh in India and Xinjiang in China (Schaller et al. 1987; Fox et al. 1991; Hussain 2000; Namgail et al. 2007; Li et al. 2013). It would be interesting to know why the values reported by Alexander et al. (2015) are so small, as currently there is no indications of why.

Our questionnaire did not record any surplus killing (mass killing that ranges from 25–150 domestic goats/sheep when snow leopards enter a corral/pen). However, in September 2015, a kill of 104 mountain goats by snow leopards was reported in Charang in Upper Mustang (Fig. 8.4). Although these incidents account for only 14% of all the reported killings (n = 210), they account for nearly 50% of all the livestock killed in Hemis National Park, India (Jackson and Wangchuk 2001). Other studies also report mass killing by both snow leopards and wolves in the Pamir mountains (Hussain 2000; Mishra and Fitzherbert 2004; Watanabe et al. 2010). Thus, surplus killing, although not recorded in this study, cannot be neglected.

In the area studied, other predators such as wolf, red fox, golden jackal and lynx killed fewer livestock than snow leopards. This may be because Himalayan wolf, which was thought to have been exterminated by herdsmen three decades ago, has only recently reappeared in ACA (Chetri et al. 2016; Shrestha 2016). These findings differ from those of other studies. For example, lynx or wolf-related mortality of livestock is reported to exceed three to four times that of snow leopards in QNNR and Xinjiang in China and Ladakh in India (Namgail et al. 2007; Li et al. 2013; Alexander et al. 2015).

The economic value of livestock killed by predators was $29 per household per year in 1990 in UM (Oli et al. 1994), while in 2015 this study revealed it was higher by an order of magnitude ($349 per household per year). This can be attributed to both increases in the number killed and their value. It is a significant loss when compared with the per capita annual income, which was US$ 200 in 1990 and US$ 750 in 2015. Over the period 1990 to 2007, annual economic losses associated with predation ranged from US$ 29 to US$ 300 per household, a significant sum given per capita annual incomes of US$ 250–400 (Oli et al. 1994; Jackson et al. 1996; Mishra 1997; Ikeda 2004; Namgail et al. 2007). After 2007, the annual economic loss was US$ 80–650 (Li et al. 2013; Din et al. 2017). Although the predator-induced loss was substantial, all these studies report that livestock mortality due to other causes such as diseases etc. was higher than that due to predators.

Fig. 8.4 Surplus killing of mountain goats by snow leopards in Charang (Upper Mustang, Nepal) in 2015 (Photo by Niraj Thakali)

In Lupra in LM and Phortse in in SNP, respondents were more negative in their attitude towards snow leopards and all kills were attributed to them, even though common leopards were present in the habitat at 4000 m near to the forest line. However, there are many factors, which might have biased the results of the questionnaire:

(i) herders' perception of specific species as causative agents or exaggerated reporting of herders (Suryawanshi et al. 2013),

(ii) a kill may be attributed to a predator, even though it was only scavenging the carcass of an animal that had died for other reasons (Oli et al. 1994),

(iii) misidentification of predators when snow leopards are confused with other large carnivores like wolf, lynx and common leopard (Mishra 1997),

(iv) behaviour of specific carnivores in their interactions with humans may influence perceptions concerning the species (Lescureux and Linnell 2010); for example, the greater visibility and howling of wolves may heighten the perception of the risk they pose to livestock (Kellert et al. 1996),

(v) reports of predation by snow leopards might have been subject to a "social desirability" bias, as snow leopards are recognized as an iconic, protected species (Li et al. 2013); however, information on how the local people view predation will facilitate the development of a programme for mitigating human-wildlife conflicts and conserving snow leopards.

8.5.2 Livestock Herding

The present study showed that the abundance of large livestock has increased in UM and that of goats/sheep has increased in LM and UM. As indicated above, the variation in livestock mortality due to snow leopards may be due to both changes in the abundance of livestock and guarding practices in different regions or different years. Herding is just one factor influencing the number of attacks on livestock by predators. The most important are livestock abundance (Suryawanshi 2013; Zarco-González et al. 2013), herd composition (Tortato et al. 2015), poor care of calves and improper carcass disposal (Pena-Mondragon et al. 2017), guarding (Kolowski and Holekamp 2006; Hemson et al. 2009) and patterns of rotation (Kissling et al. 2009).

8.5.3 Local People's Perception of Snow Leopards and Suggested Mitigation Measures

In 1990 in UM, 52% of the respondents agreed that the elimination of snow leopards is the only viable option, while only 10% suggested compensation would be a satisfactory solution (Oli et al.1994). This study revealed that the level of conflict has decreased slightly, but 15% of the respondents still had a negative attitude towards snow leopards (Fig 8.2). Killing of snow leopard (especially those that attack livestock) was also ranked as the second or third of the five most appropriate mitigation measures (Fig. 8.3). Other studies report similar results: local people consider eradication of predators as a possible solution in the Spiti region in Indian Trans-Himalaya (Bagchhi 2005) and in Tajik in Afghan and Pakistan Pamir (Din et al. 2017). In contrast, Suryawanshi et al. (2014) report that most people have a positive attitude to snow leopards in the Spiti Valley in India, even if they suffer high levels of livestock loss due to predators. This may be due to their Buddhist faith and 15 years of conservation in this region. Our study also showed the effects of similar religious beliefs regarding snow leopards (Buddhist culture) in the areas studied. However, this tolerance of predators diminishes when

the killing of livestock reaches a level when it becomes too high for the herders to survive in the absence of an alternative means of livelihood and income.

Fig. 8.5 Fur hat made from pelt of a predator in a house in Upper Manang in 2016

8.5.4 Threats to the Conservation of Snow leopards

In the three areas studied, the lowest number of severe threats due to human related activities were recorded in SNP. The threat reduction assessment index was higher in SNP than in the other areas studied, but could be improved by an intensification of the conservation programme. This indicates that there is further work to be done to improve on the level of conservation of snow leopards and their prey in all three areas studied. In addition to these threats to conservation, the weak enforcement of the law is still a problem in UM. During the period of the survey, 2014–2016, the local people were still wearing fur hats made from fox and jackal pelts despite hunting being prohibited (Fig. 8.5).

We conclude that the conflict between snow leopards and people in the three areas studied is still major threat to the long-term survival of snow leopards. The mitigation measures that were recommended after consultation with the local people would go a long way in safeguarding the livelihoods of the local people and conserving snow leopards.

Contribution

DBR carried out the household survey in UM supervised by BS and JBK. The rest of the data were collected by BS. BS wrote the original draft and BS, TBK and PK completed and reviewed the manuscript.

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